AR201-14066A

HIGH PRODUCTION VOLUME (HPV)

CHALLENGE PROGRAM

TEST PLAN

For

ZINC DIALKYLDITHIOPHOSPHATE CATEGORY

Prepared by
The American Chemistry Council
Petroleum Additives Panel
Health, Environmental, and Regulatory Task Group

September 24, 2002

LIST OF MEMBER COMPANIES IN THE HEALTH, ENVIRONMENTAL AND REGULATORY TASK GROUP

The Health, Environmental, and Regulatory Task Group (HERTG) of the American Chemistry Council Petroleum Additives Panel includes the following member companies:

BP plc

Chevron Oronite Company, LLC

Crompton Corporation

Ethyl Corporation

ExxonMobil Chemical Company

Ferro Corporation

Infineum

The Lubrizol Corporation

Rhein Chemie Corporation

Rhodia, Inc. (formerly Albright & Wilson Americas Inc.)

EXECUTIVE SUMMARY

The American Chemistry Council Petroleum Additives Panel Health, Environmental, and Regulatory Task Group (HERTG), and its member companies, hereby submit for review and public comment their test plan for the *"Zinc dialkyldithiophosphate"* category of chemicals under the United States Environmental Protection Agency High Production Volume (HPV) Chemical Challenge Program. This report should be read in its entirety in order to obtain a complete understanding of the chemical category and proposed testing.

Zinc dialkyldithiophosphate Category. Relying on several factors specified in the EPA guidance document on "Development of Chemical Categories in the HPV Challenge Program," in which use of chemical categories is encouraged, the following twelve closely related chemicals constitute a chemical category:

- Phosphorodithioic acid, mixed O,O-bis(1,3-dimethylbutyl and iso-propyl) esters, zinc salts – (CAS # 84605-29-8), referred to as "mixed isopropyl and 1,3-dimethylbutyl derivative"
- Phosphorodithioic acid, mixed O,O-bis(iso-butyl and pentyl) esters, zinc salts – (CAS # 68457-79-4), referred to as "mixed isobutyl and pentyl derivative"
- Phosphorodithioic acid, mixed O,O-bis(sec-butyl and 1,3-dimethylbutyl) esters, zinc salts – (CAS # 68784-31-6), referred to as "mixed sec-butyl and 1,3-dimethylbutyl derivative"
- Phosphorodithioic acid, mixed O,O-bis(sec-butyl and isooctyl) esters, zinc salts – (CAS # 113706-15-3), referred to as "mixed sec-butyl and isooctyl derivative"
- Phosphorodithioic acid, O-(2-ethylhexyl) O-isobutyl ester, zinc salt – (CAS # 26566-95-0), referred to as "mixed isobutyl and 2-ethylhexyl derivative"
- Phosphorodithioic acid, mixed O,O-bis(iso-butyl and isooctyl and pentyl) esters, zinc salts – (CAS # 68988-46-5), referred to as "mixed isobutyl, pentyl and isooctyl derivative"
- Phosphorodithioic acid, O,O-bis(1,3-dimethylbutyl) ester, zinc salt – (CAS # 2215-35-2), referred to as "1,3-dimethylbutyl derivative"
- Phosphorodithioic acid, O,O-bis(2-ethylhexyl) ester, zinc salt – (CAS# 4259-15-8), referred to as "2-ethylhexyl derivative"
- Phosphorodithioic acid, O,O-bis(isooctyl) ester, zinc salt – (CAS# 28629-66-5), referred to as "isooctyl derivative"
- Phosphorodithioic acid, O,O-diisodecyl ester, zinc salt – (CAS # 25103-54-2), referred to as "diisodecyl derivative"
- Phenol, dodecyl-, hydrogen phosphorodithioate, zinc salt – (CAS # 54261-67-5), referred to as "dodecylphenol derivative"
- Phenol, tetrapropenyl-, hydrogen phosphorodithioate, zinc salt – (CAS # 11059-65-7), referred to as, "tetrapropenylphenol derivative".

Although the TSCA Inventory Update Rule (IUR) reports list 1990 production volumes for "Phenol, dodecyl (*straight-chain* C12)-, hydrogen phosphorodithioate, zinc salt" (CAS # 54261-67-5), it is typical for additive manufacturers to synthesize the *branched* C12 tetrapropenyl (CAS # 11059-65-7) congener using propylene tetramer. Propylene tetramer is a distilled product manufactured from oligomerization of 1-propene under acid catalysis conditions. Commercial propylene tetramer is a range of C10-C15 olefins with the C12 propylene tetramer isomer being ~60 wt-% of the total. Although study reports may identify the dodecyl derivative as the test article, the tetrapropenyl derivative is and always has been the prepared chemical. Therefore, in this test plan the presented data for CAS registry numbers 54261-67-5 and 11059-65-7 should be considered interchangeable and referred to as the same chemical species.

Structural Similarity. A key factor supporting the classification of these chemicals as a category is their structural similarity. Zinc dialkyldithiophosphates are used as multi-functional anti-wear and anti-oxidation inhibitor performance components in passenger motor oils, diesel engine oils and industrial oils such as hydraulic lubricants. All substances in this category consist of alkyl (C3-C12) or alkaryl (C12 alkylphenol) substituted phosphorodithioic acid structures complexed with zinc. Zinc dialkyldithiophosphates are manufactured and distributed in commerce in highly refined lubricant base oil (IP 346 DMSO extractables < 3%). The oil is added during the neutralization of the dithiophosphate alkyl esters intermediate with zinc oxide. The oil acts as a solvent in the reaction, manages the viscosity and improves consistency of the final product. The zinc dialkyldithiophosphates are never isolated from base oil at any time during their life cycle. Hence, all testing for environmental fate, aquatic toxicity and health effects was performed on zinc dialkyldithiophosphates in highly refined lubricant base oil.

Similarity of Physicochemical Properties. The similarity of the physicochemical properties of these substances parallels their structural similarity. Zinc dialkyldithiophosphates are amber colored viscous liquids containing 10-15 wt-% highly refined lubricating base oil (representative lubricating base oil CAS registry numbers are 64742-54-7 and 64741-88-4). The physicochemical properties of zinc dialkyldithiophosphates largely reflect those of base oil. These materials are relatively high molecular weight, low vapor pressure, high viscosity, and poorly water soluble

Fate and Transport Characteristics. The zinc dialkyldithiophosphates are formulated for use in oils and have low water solubility. Solubility testing will be conducted on representative low and high molecular weight members of this category to confirm available data. Members of this category have been shown to be poorly biodegradable. Adequate biodegradation data exist for two commercial oil-based samples of the zinc dialkyldithiophosphate category. Bridging will be used to fill the remaining data gaps for the other ten substances. Available literature and historical information indicates that these materials are stable and are not susceptible to hydrolysis under normal conditions. These materials are known to be thermally labile at temperatures >120^{o}C. This decomposition mechanism is key to how they provide anti-wear and anti-oxidation performance enhancements in engine oils. A search of the chemical literature has shown no known photochemical pathways, therefore photodegradation is not expected to cause significant physical degradation of zinc dialkyldithiophosphates. Nevertheless, the UV absorption data will be collected on 2 representative members of the category. If feasible, first order reaction rates will also be calculated for chemicals identified to have a potential for direct photolysis in water.

Toxicological Similarity. The zinc dialkyldithiophosphates have a long history of use in lubricants and published and unpublished aquatic toxicity data is available for many of the members in this category. There is a wide variability in results among closely related members and even for the same chemical in this category. There are many contributing factors for this apparent variability in data, which are discussed later in this document. Review of existing published and unpublished mammalian toxicity test data for commercial samples of zinc dialkyldithiophosphates in highly refined lubricant base oil suggest that the toxicity profiles of these materials are similar. Data obtained from the proposed additional testing will further characterize of the toxicity endpoints in the HPV Challenge Program for all members within this category.

Aquatic Toxicology. Acute fish, invertebrate, and alga toxicity data for zinc dialkyldithiophosphates in highly refined lubricant base oil were reviewed, and the findings show a large variability in data. Additional testing is proposed to characterize the aquatic toxicity potential for members of this category.

Mammalian Toxicology - Acute. Data on acute mammalian toxicity of zinc dialkyldithiophosphates in highly refined lubricant base oil were reviewed, and the findings indicate a low concern for acute toxicity. No additional acute mammalian toxicity testing is proposed.

Mammalian Toxicology - Mutagenicity. Data from bacterial reverse mutation assays, *in vitro* mutation assays in mammalian cells and *in vivo* chromosome aberration studies were reviewed. Findings indicate that commercial samples of zinc dialkyldithiophosphates in highly refined lubricant base oil have a low potential for inducing genetic toxicity. Due to the similarity of structure and physicochemical properties, the existing data can be bridged to the other members of the category where information is lacking. As a result, the category is adequately tested for mutagenicity, and no additional collection of genetic toxicity data is proposed.

Mammalian Toxicology - Systemic Toxicity. Data from several repeated-dose toxicity studies using commercial samples of zinc dialkyldithiophosphates in highly refined lubricant base oil were reviewed. Repeated dermal exposure to experimental animals resulted in moderate-to-severe dermal irritation, behavioral distress, body weight loss and emaciation, reduction in hematological parameters and adverse effects on male reproductive organs. Oral administration caused significant gastric irritation and related gastrointestinal disturbances, signs of distress but with no evidence of adverse effects on male reproductive organs. Bridging will be used to satisfy repeated dose data gaps for those zinc dialkyldithiophosphates in highly refined lubricant base oil where the carbon chain lengths/molecular weights are similar, yet lack subchronic toxicity information. No additional repeated-dose systemic toxicity testing is proposed.

Mammalian Toxicology - Reproductive and Developmental Toxicity. Data from a study on the 2-ethylhexyl derivative in highly refined lubricant base oil indicates a low concern for reproduction/ developmental toxicity. Furthermore, an epidemiological study on workers exposed to oil-based zinc dialkyldithiophosphates (range C4-C8) in an additive manufacturing plant revealed no adverse effects on worker reproductive health. Review of the available information underscores the similarity of clinical and pathological findings in repeated-dose dermal toxicity studies with C4-C10 zinc dialkyldithiophosphates, as well as the absence of reproduction and developmental toxicity and the lack of untoward findings in a human epidemiological investigation. In light of the irritant properties that zinc dialkyldithiophosphates in highly refined lubricant base oil have on skin and gastrointestinal mucosa, any additional repeated dose testing would cause unnecessary distress and suffering in experimental animals, and would add no additional insight into the hazard assessment of this category of substances. Therefore, the HERTG concludes that the existing information is adequate to characterize the reproduction/developmental toxicity profile of the entire zinc dialkyldithiophosphate category. No additional repeated dose testing is proposed.

Conclusion. Based upon the data reviewed for this test plan, the physicochemical, environmental fate, and toxicological properties of the proposed zinc dialkyldithiophosphate category members in highly refined lubricant base oils are similar and/or follow a regular, predictable pattern. Variability was seen in the aquatic toxicity data between the same or closely related members of the category. This is attributed to the test methodology used and is not due to a difference in the nature or chemistry of the category members. Further testing is therefore proposed to confirm this hypothesis. Therefore, the EPA definition of a chemical category has been met, and the twelve chemicals that constitute the zinc dialkyldithiophosphate category will be tested in accordance with the test plan summarized below.

Test Plan. The test plan for the zinc dialkyldithiophosphate category includes the following tests or computer modeling:

- Water solubility – The solubility of the mixed isopropyl and 1,3-dimethyl derivative (CAS# 84605-29-8), 2-ethylhexyl derivative (CAS # 4295-15-8) and the tetrapropenylphenol derivative (CAS# 11059-65-7) will be measured. Results will be bridged to other members of the category.
- Photodegradation– UV absorption data will be collected on the mixed isopropyl and 1,3-dimethyl derivative (CAS# 84605-29-8) and the isooctyl derivative (CAS# 28629-66-5) to determine whether there is a potential for direct photodegradation.
- Fugacity modeling – Environmental partitioning data for members of this category will be calculated using a Mackay Level I equilibrium partitioning model and provided in robust summaries.
- Acute fish toxicity – Tests will be conducted on mixed isopropyl and 1,3-dimethylbutyl derivative (CAS# 84605-29-8), 2-ethylhexyl derivative (CAS# 4259-15-8), and tetrapropenylphenol derivative (CAS# 11059-65-7). Results will be bridged to other members of the category.
- Acute invertebrate toxicity – Tests will be conducted on mixed isopropyl and 1,3-dimethylbutyl derivative (CAS# 84605-29-8), 2-ethylhexyl derivative (CAS# 4259-15-8),

and tetrapropenylphenol derivative (CAS# 11059-65-7). Results will be bridged to other members of the category.

- Alga toxicity - Tests will be conducted on mixed isopropyl and 1,3-dimethylbutyl derivative (CAS# 84605-29-8), 2-ethylhexyl derivative (CAS# 4259-15-8), and tetrapropenylphenol derivative (CAS# 11059-65-7). Results will be bridged to other members of the category.

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1.0 INTRODUCTION

In March 1999, the American Chemistry Council (formerly the Chemical Manufacturers Association) Petroleum Additives Panel Health, Environmental, and Regulatory Task Group (HERTG), and its participating member companies committed to address data needs for certain chemicals listed under the Environmental Protection Agency (EPA) High Production Volume (HPV) Chemical Challenge Program. This test plan follows up on that commitment.

Specifically, this test plan sets forth how the HERTG intends to address physico-chemical, environmental, aquatic and health effects testing information for the following twelve substances:

- Phosphorodithioic acid, mixed O,O-bis(1,3-dimethylbutyl and iso-propyl) esters, zinc salts – (CAS # 84605-29-8), referred to as "mixed isopropyl and 1,3-dimethylbutyl derivative"
- Phosphorodithioic acid, mixed O,O-bis(iso-butyl and pentyl) esters, zinc salts – (CAS # 68457-79-4), referred to as "mixed isobutyl and pentyl derivative"
- Phosphorodithioic acid, mixed O,O-bis(sec-butyl and 1,3-dimethylbutyl) esters, zinc salts – (CAS # 68784-31-6), referred to as "mixed sec-butyl and 1,3-dimethylbutyl derivative"
- Phosphorodithioic acid, mixed O,O-bis(sec-butyl and isooctyl) esters, zinc salts – (CAS # 113706-15-3), referred to as "mixed sec-butyl and isooctyl derivative"
- Phosphorodithioic acid, O-(2-ethylhexyl) O-isobutyl ester, zinc salt – (CAS # 26566-95-0), referred to as "mixed isobutyl and 2-ethylhexyl derivative"
- Phosphorodithioic acid, mixed O,O-bis(iso-butyl and isooctyl and pentyl) esters, zinc salts – (CAS # 68988-46-5), referred to as "mixed isobutyl, pentyl and isooctyl derivative"
- Phosphorodithioic acid, O,O-bis(1,3-dimethylbutyl) ester, zinc salt – (CAS # 2215-35-2), referred to as "1,3-dimethylbutyl derivative"
- Phosphorodithioic acid, O,O-bis(2-ethylhexyl) ester, zinc salt – (CAS# 4259-15-8), referred to as "2-ethylhexyl derivative"
- Phosphorodithioic acid, O,O-bis(isooctyl) ester, zinc salt – (CAS# 28629-66-5), referred to as "isooctyl derivative"
- Phosphorodithioic acid, O,O-diisodecyl ester, zinc salt – (CAS # 25103-54-2), referred to as "diisodecyl derivative"
- Phenol, dodecyl-, hydrogen phosphorodithioate, zinc salt – (CAS # 54261-67-5), referred to as "dodecylphenol derivative"
- Phenol, tetrapropenyl-, hydrogen phosphorodithioate, zinc salt – (CAS # 11059-65-7), referred to as, "tetrapropenylphenol derivative".

Although the TSCA Inventory Update Rule (IUR) reports list 1990 production volumes for "Phenol, dodecyl (*straight-chain* C12)-, hydrogen phosphorodithioate, zinc salt" (CAS # 54261-67-5), it is typical for additive manufacturers to synthesize the *branched* C12 tetrapropenyl congener (CAS # 11059-65-7) using propylene tetramer. Therefore, the tetrapropenyl derivative is included in this category analysis document and testing plan so that the physicochemical, environmental fate, aquatic toxicity and health effects of all high production volume zinc dialkyldithiophosphates in highly refined lubricant base oil will be captured. Consequently, data submitted in this category justification and testing plan for CAS # 54261-67-5 and 11059-65-7 should be considered interchangeable and referred to as the same chemical species.

An analysis of the available data on these chemicals supports the designation of the zinc dialkyldithiophosphates as a "chemical category" as provided in the EPA guidance document entitled, "Development of Chemical Categories in the HPV Challenge Program". This document provides the basis for that determination, indicates the findings of the data review process, and sets forth a proposed testing plan to satisfy parts of the required test battery for endpoints without data that would be considered adequate under the program.

EPA guidance on the HPV Chemical Challenge Program indicates that the primary purpose of the program is to encourage "the chemical industry . . . to voluntarily compile a Screening Information Data Set (SIDS) on all chemicals on the US HPV list." (EPA, "Development of Chemical Categories in the HPV Challenge Program," p. 1) At the same time, EPA recognizes that the "large number of chemicals to be tested [about 2800 HPV chemicals] makes it important to reduce the number of tests to be conducted, *where this is scientifically justifiable.*" (*Id.*, p. 1) [emphasis added] The next part of the guidance explains where this would be scientifically justifiable:

> One approach is to test closely related chemicals as a group, or category, rather than test them as individual chemicals. In the category approach, *not every chemical needs to be tested for every SIDS endpoint.* However, *the test data finally compiled* for the category must prove adequate to support a screening level hazard-assessment of the category and its members. That is, the *final data set* must allow one to estimate the hazard for the untested endpoints, *ideally* by interpolation between and among the category members. In certain cases, where toxicity is low and no upward trend is expected, extrapolation to the higher category members may be acceptable. (*Id.*, p. 1) [emphasis added].

EPA guidance goes on to state, "The use of categories is encouraged in the Challenge Program and will have a number of benefits." (*Id.,* p. 1) Among the benefits identified in the guidance for the use of categories are "a reduction in testing will result in fewer animals used to test a category of chemicals as opposed to doing each test on each individual chemical," and "there will be . . . economic savings since less testing may be needed for chemicals considered as a category." (*Id.*, p. 1) That guidance also states that categories "accomplish the goal of the Challenge Program – to obtain screening level hazard information – through the strategic application of testing to the category." (*Id*, p. 2)

A similarly stated intent "to reduce the number of tests to be conducted, *where this is scientifically justifiable*" was articulated by the Agency in its draft guidance document titled, "The Use of Structure Activity Relationships (SAR) in the High Production Volume Chemicals Challenge Program." [emphasis added].

The EPA “Chemical Categories” guidance sets forth a definition of what constitutes a “chemical category, for the purposes of the Challenge Program”. Specifically, that definition states that a chemical category under the HPV Challenge Program “is a group of chemicals whose physicochemical and toxicological properties *are likely to* be similar *or* follow a regular pattern as a result of structural similarity.” (*Op. Cit*., p. 2) [emphasis added].

According to the guidance, what is important is that the “structural similarities [among members of the group] *may* create a predictable pattern *in any* or all of the following parameters: physicochemical properties, environmental fate and effects, and human health effects.” (Id., p. 2) [emphasis added]. Thus, it is not necessary for the chemicals in a category to be similar in all respects. Nor must there be conclusive proof that the chemicals in the postulated category will behave identically across all relevant parameters. All that is required for an acceptable category under the HPV Challenge Program is that there be a *likelihood* of similarity of physicochemical and toxicological properties or a *likelihood* that the chemicals will in some pertinent respect follow a regular pattern as a result of their structural similarity.

In identifying the zinc dialkyldithiophosphate category, the six-step process set out in the EPA guidance on category development was followed. As the information below indicates, the zinc dialkyldithiophosphate chemicals clearly satisfy the standards established in that guidance for use of a chemical category:

Step 1: group structurally similar chemicals into a putative category

Step 2: gather relevant published and unpublished literature for each member of the category

Step 3: evaluate the compiled data for adequacy in accordance with the EPA guidance documentation

Step 4: construct matrices of SIDS endpoints versus category members arranged so as to indicate the structural progression of the category (in this case, by increasing molecular weight)

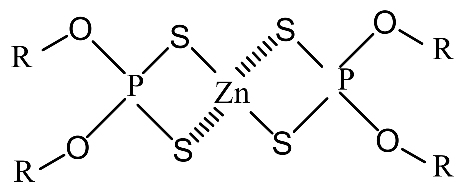
Step 5: evaluate the data to determine whether there is a correlation between category members for each SIDS endpoint

Step 6: make available to EPA, and to the public for review, this test plan including the foregoing category definition and rationale and the following data assessment with the proposed testing scheme for the zinc dialkyldithiophosphates.

2.0 CHEMISTRY OF ZINC DIALKYLDITHIOPHOSPHATES

2.1 Description

Zinc dialkyldithiophosphates consist of a phosphorodithioic acid structure with alkyl or alkaryl ester substituent groups. The alkyl groups are saturated hydrocarbon chains that vary in length (C3-C10) and in the extent of branching. An idealized structure for the zinc dialkyldithiophosphate component is shown below.



R = C3 – C10 (linear and/or branched) alkyl or C12 (branched) alkaryl

The chemical names and CAS numbers for the members of the zinc dialkyldithiophosphate category are presented in Table 1 and the chemical structures in Table 2. These substances are prepared by reacting phosphorous pentasulfide (P_2S_5) with one or more primary or secondary C3-C10 branched or linear alcohols to form the phosphorodithioic acid ester. The only exception is the alkaryl dithiophosphate where the alcohol moiety is tetrapropenylphenol. The dithiophosphoric acid ester is further diluted with 10-15 wt-% highly refined lubricating base oil (typical CAS #s 64742-54-7 and 64741-88-4) before it is neutralized with zinc oxide. The oil acts as a solvent in the neutralization reaction, manages the viscosity of the final product and improves consistency. The zinc complex that is formed upon neutralization is not a salt in the traditional sense, since the Zn-S bond is more coordinate covalent in character than ionic.

It should be noted that additive manufacturers synthesize the *branched* alkaryl C12 tetrapropenyl congener using propylene tetramer. Propylene tetramer is a distilled product manufactured from oligomerization of 1-propene under acid catalysis conditions. Commercial propylene tetramer is a range of C10-C15 olefins with the C12 propylene tetramer isomer being ~60 wt-% of the total. Although study reports may identify the dodecyl derivative as the test article, the tetrapropenyl derivative is and always has been the prepared chemical.

2.2 Physiochemical Properties

The physicochemical properties of the members of the zinc dialkyldithiophosphate category are presented in Table 3. Zinc dialkyldithiophosphate produced for use as lubricating petroleum additive are manufactured and distributed in 10-15 wt-% highly refined lubricating base oils. The highly refined lubricating base oil used in the manufacture of the zinc dialkyldithiophosphates cannot be removed without altering the structural and physicochemical character of the zinc dialkyldithiophosphate molecules. Therefore, many of the physicochemical properties presented are qualitative estimates.

2.2.1 Alkyl Chain Length and Molecular Weight

As discussed above, the members of the zinc dialkyldithiophosphate category contain alkyl chain lengths that range from C3-C10, or tetrapropenylphenol (range = C10-C15, C12 enriched). It is common for zinc dialkyldithiophosphates to contain mixed alkyl esters (e.g., C4, C5), although derivatives with single chain lengths (e.g., C8) are included in the category. Alkyl groups can be linear or branched. Two members of the category contain alkylphenol ester side-chains. As a result of this diversity in alkyl side chain length, the molecular weight distribution for the members of the category is broad, 578 to 1303 gm/mol. Due to the predominant influence of carbon chain length on molecular weight, the members of the category are arrayed in all tables in order of increasing carbon chain length.

2.2.2 Melting Point and Boiling Point

Zinc dialkyldithiophosphates as manufactured and distributed in commerce in highly refined lubricating base oil, are high viscosity liquids at ambient temperature. However, at elevated temperatures (> 120ºC), zinc dialkyldithiophosphates become unstable and degrade.

2.2.3 Vapor Pressure

Due to the technical difficulty in isolating intact zinc dialkyldithiophosphates from the highly refined lubricating base oil, vapor pressure has not been measured on the pure chemical. However, vapor pressure measurements have been performed by a consortium member company on C8 ester zinc dialkyldithiophosphate (90% in base oil) and on the pure highly refined lubricating base oil. A vapor pressure of < 0.5 mmHg at 25^{o}C and 60^{o}C was measured for both the materials. This suggests that the vapor pressure for the zinc dialkyldithiophosphate is less than 0.5 mmHg.

2.2.4 Water Solubility and Octanol-Water Partition Coefficients

The zinc dialkyldithiophosphates are formulated for use in oils and have very low water solubility. Unpublished company data for a commercial zinc dialkyldithiophosphate with an alkyl group less than C8 indicates a water solubility of 1.6 mg/L[1]. Historically, the zinc dialkyldithiophosphates are

[1] Information Review: Zinc Dialkyl Dithiophosphates. CRCS, Inc. Prepared under EPA Contract No. 68-01-6650 for TSCA Interagency Testing Committee. October 31, 1984.

generally regarded to be poorly soluble in water. In order to adequately define the solubility range of the members of this category, water solubility testing will be conducted on selected members of this category:

- the mixed isopropyl and 1,3-dimethylbutyl derivative (CAS# 84605-29-8) in highly refined lubricating base oil, which contains the lowest molecular weight substance and the shortest alkyl side chain (C3) in the category. This is expected to be the most soluble member and should provide the upper-bound solubility value for this category
- the 2-ethylhexyl derivative (CAS # 4259-15-8) in highly refined lubricating base oil which represents a higher molecular weight member of this category
- the tetrapropenylphenol derivative (CAS # 11059-65-7) in highly refined lubricating base oil which represents a higher molecular weight alkaryl member of this category.

Unpublished company data on a commercial zinc dialkyldithiophosphate with a carbon chain length of less than eight yielded a log P value of 2.49[1]. Longer chain materials are likely to have higher octanol/water partition coefficients. The log P is a measure of the lipophilicity of a substance and is used as a surrogate indicator of the potential of a chemical substance to bioaccumulate in aquatic organisms. While Log P is a good predictor of bioaccumulation for nonpolar organic compounds, the mechanisms for uptake and depuration of metals and metal compounds are very complex and variable. For metal compounds, the Log P data are not indicative of the bioaccumulation potential. In view of the above, no further testing for log P is proposed.

3.0 USES OF ZINC DIALKYLDITHIOPHOSPHATES

Zinc dialkyldithiophosphates are used to formulate finished lubricating oils including all types of automotive and diesel engine crankcase, industrial oils and hydraulic fluids. They are used as anti-wear inhibitors to reduce wear in engines and hydraulic equipment parts, and also act as antioxidants. Zinc dialkyldithiophosphates are generally sold to finished oil blenders in additive packages, where the concentration ranges from 1 to 20 wt-%. These additive packages are then blended into finished oils where the typical concentration of zinc dialkyldithiophosphate ranges from 0.1 to 10 wt-% in the finished oil.

Zinc dialkyldithiophosphates are manufactured and blended into additive packages at plants owned by members of the HERTG. Finished lubricants are blended at facilities owned by our customers. Additive packages are shipped to customers in ships, iso-containers, railroad tank cars, tank trucks or in 55-gallon steel drums. The additive packages are stored in bulk storage tanks at the customer blending sites. Finished oils are blended by pumping the lubricating oil blend stocks and the additive package from their storage tanks through computer controlled valves that meter the precise delivery of the components into a blending tank. After blending, the finished lubricant products are sold in bulk and shipped in tank trucks to large industrial users, such as manufacturing facilities and facilities that service truck fleets and passenger motor vehicles. Finished lubricants are also packaged into 55-gallon drums, 5-gallon pails, and one-gallon and one-quart containers for sale to smaller industrial users. Sales of lubricants in one-gallon and one-quart containers to consumers at service stations or retail specialty stores also occur.

Based on these uses, the potentially exposed populations include (1) workers involved in the manufacture of zinc dialkyldithiophosphates, blending them into additive packages, and blending the additive packages into finished lubricants; (2) quality assurance workers who sample and analyze these products to ensure that they meet specifications; (3) workers involved in the transfer and transport of zinc dialkyldithiophosphates, additive packages or finished lubricants that contain them; (4) mechanics who may come into contact with both fresh and used lubricants while working on engines or equipment; (5) gasoline station attendants and consumers who may periodically add lubricating oil to automotive crankcases; and (6) consumers who may change their own automotive engine oil. The most likely route of exposure for these substances is skin and eye contact. Manufacturing, quality assurance, and transportation workers will likely have access to engineering controls and wear protective clothing to minimize exposure. Mechanics wear protective clothing, but often work without gloves or eye protection. Gasoline station attendants and consumers often work without gloves or other protective equipment. The most likely source of environmental exposure is accidental spills at manufacturing sites and during transport.

4.0 EVALUATION OF AVAILABLE PUBLIC AND COMPANY DATA

4.1 Environmental Fate Data

4.1.1 Physicochemical Properties Relevant to Environmental Fate

In order to understand the environmental fate of a substance, one must understand how that substance can potentially partition among environmental compartments (i.e., air, soil, sediment, suspended sediment, water, and biota). The physicochemical properties of a substance influence the way in which a substance will degrade. The important environmental degradation pathways include biodegradation, hydrolysis, and photodegradation. Biodegradation is a measure of the potential of a compound to be degraded by microorganisms. Hydrolysis is a reaction in which a water molecule or hydroxide ion substitutes for another atom or group of atoms present in an organic molecule. Photodegradation is the degradation of a chemical compound as a result of absorption of solar radiation.

The physicochemical properties of the parent substance will influence the way in which these substances may partition among environmental compartments. Substances characterized by a low vapor pressure do not partition into air to any great extent. Similarly, substances that are characterized by low water solubility do not partition extensively into water. Substances that do not partition into air and water to any great extent tend to partition into soil and sediments.

4.1.2 Biodegradability

4.1.2.1 Test Methodologies

Chemical biodegradation involves a series of microbially-mediated reactions that may require many kinds of microorganisms acting together to degrade the parent substance. There are several standard test methods, which measure primary degradation (i.e., loss of parent chemical) or ultimate degradation (i.e., complete utilization of the substance to produce carbon dioxide, water, mineral salts, and microbial biomass). Primary degradation can be determined analytically by measuring dissolved organic carbon (DOC) for water-soluble chemicals, infrared absorbance, or by a chemical-specific detection method. Ultimate degradation (also called mineralization) can be determined by measuring oxygen consumption or carbon dioxide evolution relative to the theoretical levels that can be achieved based on an elemental analysis of the chemical under investigation.

4.1.2.2 Summary of Available Data

Biodegradation data for commercial samples of two of the zinc dialkyldithio-phosphates in highly refined lubricating base oil are summarized in Table 4.

The biodegradability of the dodecylphenol derivative (CAS # 54261-67-5) in highly refined lubricating base oil was evaluated using the Modified Sturm Test (OECD Guideline 301B, *CO_2 Evolution Test*) and the *Manometric Respirometry Test* (OECD Guideline 301F). After 28 days in each test, the extent of biodegradation was 5.9% based on carbon dioxide evolution and 4.2% based on theoretical oxygen demand, respectively.

The Modified Sturm Test (OECD Guideline 301B, *CO_2 Evolution Test*) was used to evaluate the biodegradability of the mixed isopropyl and 1,3-dimthylbutyl derivative (CAS # 84605-29-8) in highly refined lubricating base oil. After the 28-day test, the extent of biodegradation was 5.9% based on carbon dioxide evolution.

4.1.2.3 Data Assessment and Test Plan for Biodegradability

Adequate biodegradation data exist for two of twelve substances in the zinc dialkyldithiophosphate category including the lowest molecular weight (CAS# 84605-29-8) and the highest molecular weight (CAS# 54261-67-5) members. The results indicate that these substances are poorly biodegraded irrespective of molecular weight. Therefore, these data will be used to bridge to all intermediate molecular weight category members, thereby characterizing the biodegradability of the entire category.

4.1.3 Hydrolysis

4.1.3.1 Test Methodologies

The potential for a substance to hydrolyze in water is assessed as a function of pH (OECD Guideline 111, *Hydrolysis as a Function of pH*[2]). When an organic molecule undergoes hydrolysis, a nucleophile (water or hydroxide ion) attacks an electrophile and displaces a leaving group (e.g., halogen, phenoxide).[3] Potentially hydrolyzable groups include alkyl halides, amides, carbamates, carboxylic acid esters and lactones, epoxides, phosphate esters, and sulfonic acid esters[3]. The lack of a suitable leaving group renders compounds resistant to hydrolysis and the lack of water solubility make testing for hydrolysis unfeasible.

4.1.3.2 Summary of Available Data

Zinc dialkyldithiophosphates are formulated in oil and are hydrolytically stable under normal conditions. This is documented in various studies that have been conducted to study the hydrolytic stability and hydrolysis pathways for the zinc dialkyldithiophosphates. The studies were carried out by heating the zinc dialkyldithiophosphates at 85ºC to achieve hydrolysis[4]. These substances have little, if any, potential for hydrolysis under environmentally relevant conditions.

4.1.3.3 Data Assessment and Test Plan for Hydrolysis

Since available literature information and historical use of these substances in petroleum additive formulations indicates that these materials are not subject to hydrolytic degradative mechanisms under normal conditions, no hydrolysis testing is proposed.

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[2] Organization for Economic Cooperation and Development (OECD) (1993) OECD Guidelines for Testing of Chemicals. OECD. Paris, France.
[3] W. Lyman et al. (1990) *Handbook of Chemical Estimation Methods*. Chapter 8.
[4] Burn A.J. et al. Analysis of the Hydrolytic Stability of Zinc(II) O,O-Dialkyl Dithiophosphates as a Function of the Nature of the Alkyl Groups by 31P NMR Spectroscopy. J. Chem. Soc. Perkin Trans. 2 , 1992.

4.1.4 Photodegradation

4.1.4.1 Test Methodologies

Photodegradation can occur as a result of direct and indirect mechanisms. A prerequisite for direct photodegradation is the ability of one or more bonds within a chemical to absorb ultraviolet (UV)/visible light in the 290 to 750 nm range. Light wavelengths longer than 750 nm do not contain sufficient energy to break chemical bonds, and wavelengths below 290 nm are shielded from the earth by the stratospheric ozone layer. In comparison, indirect photodegradation also requires light energy as well as a series of chemical reactions that include a reaction of the parent molecule with hydroxyl radicals.

Direct photochemical degradation occurs through the absorbance of solar radiation by a chemical substance. If the absorbed energy is high enough, then the resultant excited state of the chemical may lead to its transformation. Simple chemical structures can be examined to determine whether a chemical has the potential for direct photolysis in water. First order reaction rates can be calculated for some chemicals that have a potential for direct photolysis using the procedures of Zepp and Cline[5].

To develop information or data that will characterize the potential of products in this category to undergo direct photochemical degradation, the existing product chemical composition data will be evaluated to select a subset of chemicals that adequately represents products in this category. The selection process will consider chemical carbon number rage and structures.

4.1.4.2 Summary of Available Data

There are no published or unpublished photodegradation studies for members of the zinc dialkyldithiophosphate category. An initial review of the members of the zinc dialkyldithiophosphate category suggests that category members do no contain bonds that have a high potential to absorb UV light above 290 nm.

4.1.4.3 Data Assessment and Test Plan for Photodegradation

HPV Challenge Program guidance suggests that photodegradation testing be performed on each member of a category or adequate data used to bridge from selected category members with data to the remaining members that have not been tested. The UV light absorption spectra will be taken for the mixed isopropyl and 1,3-dimethylbutyl derivative (CAS # 84605-29-8) and the isooctyl derivative (CAS # 28629-66-5). The results obtained for these substances will be

[5]Zepp, R. G., and D. M. Cline. 1977. Rates of Direct Photolysis in the Aqueous Environment. Environ. Sci. Technol. 11:359.366.

indicative of whether direct photolysis is a relevant pathway for members of this category. If feasible, first order reaction rates will also be calculated for chemicals identified to have a potential for direct photolysis in water. The results of the calculations will be summarized in a technical discussion for this endpoint. Indirect photodegradation as a result of hydroxyl radical interaction is not a significant pathway as these substances are not volatile and will not exist in the vapor phase.

4.1.5 Fugacity Modeling

4.1.5.1 Modeling Methodologies

Fugacity-based multimedia fate modeling compares the relative distribution of chemicals among environmental compartments. A widely used model for this approach is the EQC model[6].

There are multiple levels of the EQC model. In the document, "Determining the Adequacy of Existing Data", EPA states that it accepts Level I fugacity modeling to estimate transport/distribution values. The EQC Level I model utilizes input of basic chemical properties, including molecular weight, vapor pressure, and water solubility to calculate percent distribution within a standardized environment. EQC Level III model uses these parameters to evaluate chemical distribution based on emission rates into air, water, and soil, as well as degradation rates in air, water, soil, and sediment.

4.1.5.2 Summary of Available Data

There are no published or unpublished fugacity-based multimedia fate modeling data for members of the zinc dialkyldithiophosphate category. All of the members of this category have low vapor pressure and low water solubility indicating that they will not tend to partition into the air or water to any great extent.

4.1.5.3 Test Plan for Fugacity

The relative distribution of substances within this category among environmental compartments will be evaluated using the Level I model. Data developed using a Level I model can then be used for simple comparative purposes across several substances. EQC Level III will not be used for this evaluation because appropriate emission levels are as yet unknown. Because of the physical nature of the substances in this category, a Level I data set will be as equally robust as a Level

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6. Mackay, D., A. Di Guardo, S. Paterson, and C. E. Cowan. 1996. Evaluating the Environmental Fate of a Variety of Types of Chemicals Using the EQC Model. Environ.

III data set and can then be used to assess the potential partitioning behavior of zinc dialkyldithiophosphate category members in the environment.

4.2 ECOTOXICOLOGY DATA

4.2.1 Aquatic Ecotoxicity Testing

4.2.1.1 Test Methodologies

Acute aquatic ecotoxicity tests are usually conducted with three species that represent three trophic levels in the aquatic environment: fish, invertebrates, and algae. The fish acute toxicity test (OECD Guideline 203, *Fish, Acute Toxicity Test*) establishes the lethality of a substance to a fish during a 96-hour exposure period. The acute invertebrate test (OECD Guideline 202, *Daphnia sp., Acute Immobilization Test and Reproduction Test*) establishes the lethality of a substance to an invertebrate, typically a daphnid (*Daphnia magna*), during a 48-hour exposure period. The alga growth inhibition test (OECD Guideline 201, *Alga, Growth Inhibition Test*) establishes the potential of a substance to inhibit alga growth, typically using the freshwater unicellular green algae, *Pseudokirchneriella subcapitata* (formerly called *Selenastrum capricornutum*), during a 96-hour exposure period.

Three test methodologies are commonly used to conduct aquatic toxicity tests; i.e., flow-through, static, and static renewal tests.

In *flow-through tests*, organisms are continually exposed to fresh chemical concentrations in each treatment level in the incoming water and there is greater assurance than with other test methods that the exposure levels and water quality remains constant throughout the test. Although flow-through testing is the preferred method, it is only applicable for chemicals that have adequate water solubility for testing.

In *static tests*, organisms are exposed in the test medium that is not replaced for the duration of the study. There is less assurance that the test concentrations will remain constant because test material can be adsorbed onto test chambers, degraded, volatilized, or otherwise changed during the test. Nevertheless, due to limitations of other test systems for non-volatile materials, the static test has been widely used, especially for testing organisms such as algae and *Daphnia*.

The *static-renewal test* is similar to a static test because it is conducted in still water, but the test solutions and control water are renewed periodically, usually every 24 hours. Daily test solution renewal provides a greater likelihood that the exposure concentrations will remain stable throughout the test. This is the preferred method for conducting aquatic toxicity tests for compounds such as the zinc dialkyldithiophosphate on fish. Daily renewals cannot be done in the algae test, and usually not in *Daphnia* tests, because the process of separation and replenishment would cause a discontinuity in the alga growth rate and it can stress, coat, or entrap *Daphnia* in any surface film during renewals. OECD considers the use of static test for *Daphnia* and algae, and the use of static renewal test for fish to be appropriate for testing poorly soluble chemicals like the zinc dialkyldithiophosphate provided that test solution preparation uses water accommodated fraction or water soluble fraction methods.[7]

4.2.1.2 Test Solution Preparation

Zinc dialkyldithiophosphates are poorly water-soluble substances, and it is not possible to prepare exposure solutions for aquatic toxicity testing by direct addition of measured quantities of test material to water. Two methods[8] are used to prepare solutions of poorly water-soluble materials for aquatic toxicity testing:

- *Water accommodated fraction* (WAF) – This is a method in which the test solution contains only that fraction of the test material (organic phase) which is retained in the aqueous phase after a period of stirring long enough to reach equilibrium, followed by a sufficient time (1-4 hours) for phase separation. The WAF (aqueous phase) will contain soluble components of the test material at levels that will be dependent on the test material loading (the amount of material added to the aqueous medium). The resulting WAF is used in the aquatic toxicity test. Ideally, a WAF consists of a water-soluble extract of test material, but it can also include a stable micro-emulsion or contain small amounts of suspended matter.

- *Water soluble fraction* (WSF) – This is a method in which a WAF is either filtered, centrifuged, or allowed to settle for a greater length of time (24 hours) than with the WAF method to remove suspended matter from the aqueous phase before being used in the aquatic toxicity test.

4.2.1.3 Reporting Toxicity Results

In both WAF and WSF tests, test material concentrations are expressed as loading rates (i.e., defined as the weight of test material added per unit volume of test medium during WAF or WSF preparation)[9]. For fish tests, endpoints can be

[7] Organization for Economic Cooperation and Development (OECD) (2000). Guidance Document on Aquatic Toxicity Testing of Difficult Substances and Mixtures. OECD Environmental Health and Safety Publications, Series on Testing and Assessment No.23, Paris, France.

[8] American Society for Testing and Materials (1998) D6081-98, Standard Practice for Aquatic Toxicity Testing of Lubricants: Sample Preparation and Results Interpretation.

[9] Organization for Economic Cooperation and Development (OECD) (1999) Draft Guidance document on Aquatic Toxicity Testing of Difficult Substances. OECD, France.

expressed as median lethal loading rate (LL_{50}) when lethal effects occur to 50% of the test population or in cases where no lethal effects are observed at all loadings tested, LL_0. In both cases, results can be expressed in mg/L and in studies where no lethality is observed, the result is expressed as LL_0 = the highest loading rate tested. For invertebrate and alga tests, endpoints are expressed as median effective loading rate (EL_{50}) or EL_0 in mg/L as discussed above.

Loading rates allow poorly water-soluble complex substances such as the zinc dialkyldithiophosphate to be compared to more readily soluble substances and /or pure chemicals on an equal basis. To allow comparison, the toxicity value is expressed as the amount of test material added per unit volume of water when preparing the WAF or WSF.

If test material exposure levels are analytically measured in the test, the endpoints can also be expressed as median lethal concentration (LC_{50}) or median effective concentration (EC_{50}) in mg/L. EC/LC_{50}s are often not reported because it is very difficult to accurately measure test material exposure levels that can be below 1.0 mg/L.

NOTE: In this test plan, these results are reported as loading rates (EL/LL), to reflect the current reporting practices for the WAF method used in the tests. In the robust summaries, these data are presented as concentrations (EC/LC) as originally reported even though the test methods employed WAF preparation of test solutions without measurement of test material concentration.

4.2.2 Aquatic Toxicity of the Zinc Dialkyldithiophosphates

In general, the toxicity of a substance to an organism is limited by mechanisms of uptake and movement to target organs. Characteristics such as smaller molecular size and a lesser degree of ionization increase the ability of a substance to passively cross biological membranes. However, the soluble fraction of a compound in water represents the chemical fraction responsible for toxicity to aquatic organisms. Therefore, aquatic toxicity can be limited by the water solubility of a substance.

Available data and historical use information indicates that the zinc dialkyldithiophosphates formulated in highly refined lubricating base oil have limited water solubility. . However, the length of the alkyl side chains on these substances may influence their relative water solubility, and, hence, their relative toxicity. As discussed earlier in this document, the proposed water solubility testing on selected members of this category will adequately define the solubility range of the members of this category and provide guidance for conducting aquatic toxicity tests.

4.2.2.1 Summary of Available Data

The zinc dialkyldithiophosphates have a long history of use in lubricants and published and unpublished aquatic toxicity data are available for many of the members in this category. However, there is significant variability in results among closely related members and even for the same chemical in this category. A thorough review of the available data was conducted by the HERTG and it appears that the variability in data may be a result of differences in both the testing methodology and test material used. There is little or no information available on test material purity and the amount of the active material in the sample to correlate the dose-response seen in a study to the test material.

Test methodology used in older studies appears to be a significant factor in the variability seen in the data. As discussed earlier, the zinc dialkyldithiophosphates are sparingly soluble in water and direct addition of these chemicals to the exposure solution is not feasible. In some of the studies, the test material was added directly above its water solubility resulting in the presence of undissolved test material including oil droplets and surface sheen in the exposure solution. It is apparent in some of the studies that physical fouling (coating of the fish gills and trapping of smaller invertebrates) contributed to the toxicity and erratic dose response was seen. For example, higher mortality/effects were seen in the lower test concentration compared to the higher test concentration. In other studies, even though a WAF approach was used, proper techniques for separating the soluble components in the water phase were not used resulting in oil sheen in the exposure solutions. It also appears that a very vigorous stirring technique was used in some studies that resulted in an inseparable emulsion of oil in water, which significantly contributed to adverse effects in test organisms. In some of the older studies, use of a co-solvent and dissolution of the test material above its water solubility limit may have contributed to toxicity that would not be associated with the test material under normal, environmentally relevant exposure conditions. It should also be noted that since test material composition and purity information is unavailable for these studies, use of a solvent or the exposure to the test material over the limit of solubility may have resulted in preferential dissolution of the impurities resulting in adverse effects to the test organisms.

4.2.2.2 Data Assessment and Test Plan for Acute Aquatic Ecotoxicity

As discussed above, there is significant data variability in the available aquatic toxicity data. The LL50 and/or EL50 values range from <0.1 mg/L to >100 mg/L for a single or closely related chemical within the category. HERTG has therefore determined that these data are inadequate to characterize the hazard of the members of this category.

The following aquatic toxicity testing is proposed to characterize the hazards of the category members.

A commercial sample of the mixed isopropyl and 1,3-dimethylbutyl derivative (CAS # 84605-29-8) in highly refined lubricating base oil, which contains the lowest molecular weight substance and the shortest alkyl side chain (C3) in the category, will be tested for acute aquatic toxicity to fish, invertebrates, and algae. The results of these tests will be bridged to the rest of the similar substances in the category:

- Mixed isobutyl and pentyl derivative (CAS # 68457-79-4),
- Mixed sec-butyl and 1,3-dimethylbutyl derivative (CAS # 68784-31-6),
- Mixed sec-butyl and isooctyl derivative (CAS # 113706-15-3),
- Mixed isobutyl and 2-ethylhexyl derivative (CAS # 26566-95-0),
- 1,3-dimethylbutyl derivative (CAS # 2215-35-2),
- Mixed isobutyl, pentyl and isooctyl derivative (CAS # 68988-46-5),

A commercial sample of the 2-ethylhexyl derivative (CAS # 4259-15-8) in highly refined lubricating base oil will be tested for acute aquatic toxicity in fish, invertebrate and algae. The results will be bridged to other similar members of the category; the isooctyl derivative (CAS # 28629-66-5) and the diisodecyl derivative (CAS # 25103-54-2).

- A commercial sample of the tetrapropenylphenol derivative (CAS # 11059-65-7) in highly refined lubricating base oil will be tested for acute aquatic toxicity in fish, invertebrate and algae. The results will be bridged to the dodecylphenol derivative (CAS # 54261-67-5).

4.3 MAMMALIAN TOXICOLOGY DATA

4.3.1 Acute Mammalian Toxicity of the Zinc dialkyldithiophosphates

4.3.1.1 Acute Toxicity Test Methodology

Acute toxicity studies investigate the effect(s) of a single exposure to a relatively high dose of a substance. Potential routes of exposure for acute toxicity assays include oral, dermal, and inhalation. Oral toxicity assays are conducted by administering test material to fasted animals (typically rats or mice) in a single gavage dose. Acute dermal toxicity tests are conducted by administering test material to the shaved skin on the back of the test animal (typically rats or rabbits) and allowing the test material to stay in contact with the skin application site for a specific duration (usually 24 hours). Acute inhalation toxicity assays are conducted by exposing test animals (typically rats) in a controlled atmosphere to a fixed air concentration of the test substance for a specific duration (typically 4 hours). The test material is generated as a vapor or intentionally aerosolized into respirable particles, then metered into the exposure air at the desired concentration. Preferably, inhalation toxicity studies are conducted using either nose-only or head-only exposure to minimize potential confounding effects resulting from whole-body exposure. Whole body exposure may lead to over-prediction of inhalation toxicity hazard by increasing the body-burden of the test material through skin absorption or ingestion of test material as a consequence of grooming both during and after the inhalation exposure period.

Historically, lethality is a primary end-point of concern in acute toxicity studies, and the traditional index of oral and dermal potency is the median lethal dose that causes mortality in 50 percent of the test animals (LD_{50}). In acute inhalation studies, the traditional measurement of potency is the median lethal concentration of the test material in air that causes mortality in 50 percent of the test animals (LC_{50}). In addition to lethality, acute toxicity studies also provide insights regarding potential systemic toxicity through careful observation and recording of clinical signs and symptoms of toxicity as well as through detailed examination of tissues and organ systems.

Typically, acute oral and dermal toxicity studies are conducted using a limit dose of 5000 and 2000 mg/kg body weight, respectively, and acute inhalation toxicity studies are conducted using a limit dose of 5 mg/L for 4 hours (according to OECD and EPA testing guidelines). Prior to 1990, some acute dermal toxicity studies may have used a limit dose of 5000 mg/kg. Recently, harmonized EPA testing guidelines (August 1998) have set the limit dose for both oral and dermal acute toxicity studies at 2000 mg/kg body weight, while the recommended limit concentration for acute inhalation studies has been set at 2 mg/L for 4 hours. The limit dose test method minimizes the number of animals tested by exposing a single group of animals to a large dose (the limit dose) of the test substance. A test substance that shows little or no effects at the limit dose is considered essentially nontoxic, and no further testing is needed. If compound-related mortality is observed at the limit dose, then further testing may be necessary.

4.3.1.2 Summary of Available Data

Acute toxicity data for commercial sample of zinc dialkyldithiophosphates in highly refined lubricating base oil is summarized in Table 6. Ten members of the category have been tested by either the oral or dermal route of administration and demonstrate a low order of acute toxicity.

4.3.1.2.1 Acute Oral Toxicity

Commercial oil-based samples of eight of the twelve substances in the zinc dialkyldithiophosphate category have been tested for acute oral toxicity. The acute oral LD_{50} for these studies in rats ranged from 2000-3500 mg/kg. Clinical signs observed following treatment included diarrhea, lethargy, reduced food consumption, and staining about the nose and eye. Ptosis, piloerection, ataxia and salivation were occasionally observed. The incidence and severity of these symptoms were proportional to the dose. In many cases the effects were found to be reversible during observation week 2. Necropsy findings were few in number. Lung congestion, gastrointestinal irritation and a reduction in body fat were observed in some animals. Significant necropsy findings in survivors were uncommon. Overall, the acute oral LD_{50} for these substances ranged from 2000 – 3500 mg/kg indicative of a relatively low order of lethal toxicity.

4.3.1.2.2 Acute Dermal Toxicity

Commercial oil-based samples for nine of the twelve substances in the zinc dialkyldithiophosphate category have been tested for acute dermal toxicity. The acute dermal LD_{50}s for these studies in rabbits were greater than 2000 mg/kg (limit tests). No treatment-related mortality was observed at doses ranging from 2000-8000 mg/kg. Dermal application of the test materials to abraded skin for 24 hours typically produced moderate-to-severe erythema and edema, which in some cases persisted through the 14-day observation period. Clinical signs included varying degrees of reduced food consumption, weight loss, diarrhea, lethargy, ataxia, ptosis, motor incoordination and/or loss of righting reflex. There were no remarkable gross necropsy observations. Overall, the acute dermal LD_{50} for these substances were greater than 2000 mg/kg indicative of a relatively low order of lethal toxicity.

4.3.1.3 Data Assessment and Test Plan for Acute Mammalian Toxicity

In total, seventeen adequate acute toxicity studies have been conducted commercial samples of the zinc dialkyldithiophosphate in highly refined lubricating base oil. These studies involved two species of laboratory animals (rats or rabbits); two routes of exposure (oral and dermal); and evaluated the toxicity of ten of the twelve members of the category. The substances tested ranged from those with the shortest (C3-C6) alkyl side chains (mixed isobutyl and pentyl derivative; and mixed isopropyl and 1,3-dimethylbutyl derivative) to the derivative containing diisodecyl (C10) esters. The data consistently demonstrate a low order of acute toxicity regardless of the length of the alkyl side chain. The overall low order of acute toxicity for these substances in combination with their similar chemical structure and physicochemical properties supports the scientific justification of these twelve substances as a category within the HPV Challenge Program.

Bridging will be used to fill the acute toxicity data gaps for the remaining four category members.

Acute toxicity data for the mixed isopropyl and 1,3-dimethylbutyl derivative, mixed isobutyl and pentyl derivative, mixed sec-butyl and 1,3-dimethybutyl derivative, mixed isobutyl and 2-ethylhexyl derivative can be bridged to the following category members:

- Mixed sec-butyl and isooctyl derivative

- Mixed isobutyl, pentyl, and isooctyl derivatives.

This bridging is justifiable based on the increasing length of the alkyl side chains in this range of the category (from mixed C3-C6 to mixed C4-C8) and the lack of an increasing trend in acute toxicity across the entire category.

Acute toxicity data for the 1,3-dimethylbutyl derivative, 2-ethylhexyl derivative, isooctyl derivative can be bridged to:

- diisodecyl derivative.

This bridging is also justifiable based on the increasing length of the alkyl side chains in this range of the category (from C6 to C8) and the lack of an increasing trend in acute toxicity across the entire category.

Acute toxicity data for the tetrapropenyl derivative will be bridged to:

- dodecylphenol derivative since both members of the category are characterized by C12 alkyl side chains on an aromatic ring.

By bridging these data to the four untested substances, the acute toxicity of the category has been evaluated with respect to all acute toxicity endpoints, and no additional acute toxicity testing is proposed for the HPV Challenge Program.

4.3.2 Mutagenicity of the Zinc Dialkyldithiophosphate Category

4.3.2.1 Mutagenicity Test Methodology

Genetic toxicology is concerned with the effects of substances on genetic material (i.e., DNA and chromosomes). Within genetic material, the gene is the simplest functional unit composed of DNA. Mutations are generally non-lethal, heritable changes to genes that may arise spontaneously or because of xenobiotic exposure. Genetic mutations are commonly measured in bacterial and mammalian cells. The simplest test systems measure the occurrence of a base-pair substitution mutation in which a single nucleotide is changed followed by a subsequent change in the complementary nucleotide on the other DNA strand. Frame shift mutations occur following the deletion or insertion of one or more nucleotides, which then changes the "reading frame" for the remainder of the gene or multiple genes. Genetic testing for these types of point mutations is generally accomplished by *in vitro* cellular assays for forward or reverse mutations. A forward mutation occurs when there is a detectable change in native DNA whereas a reverse mutation occurs when a mutated cell is returned to its initial phenotype. Both base-pair substitutions and frame shift mutations are routinely measured in bacterial cells by measuring the ability of a cell to acquire the capability to grow in an environment missing an essential amino acid. In these tests, a large number of cells are examined to demonstrate a significant increase in the frequencies of mutations that occur over the frequency of spontaneous mutations.

Chromosomal aberrations are large-scale numerical or structural alterations in eukaryotic chromosomes including deletions (visualized as breaks), translocations (exchanges), non-disjunction (aneuploidy), and mitotic recombination. Chromosomal breakage is the classical end point in chromosomal aberration assays. Substances that induce structural changes in chromosomes, especially chromosome breaks, are referred to as "clastogens." To visualize chromosomes and chromosomal aberrations following *in vitro* or *in vivo* treatment with a substance, cells are arrested in metaphase, treated to swell the chromosomes, fixed, transferred to slides and stained. The first metaphase following treatment is the time at which the greatest number of cells with damaged chromosomes may be observed. The most frequently used test systems investigate changes in mammalian cells (such as Chinese hamster ovary or lung cells; human or rat lymphocytes; or human, rat or mouse bone marrow cells) following either *in vitro* or *in vivo* exposure to the test substance. The micronucleus test is a common *in vivo* assay that measures the frequency of micronuclei formation (i.e., chromosomal fragments) in polychromatic erythrocytes.

4.3.2.2 Summary of Mutagenicity Data

A summary of the mutagenicity information for commercial samples of zinc dialkyldithiophosphates in highly refined lubricating base oil category is presented in Table 7. *In vitro* bacterial gene mutation assays, *in vitro* mammalian gene mutation assays, or *in vivo* chromosomal aberration assays have been conducted for seven of the twelve members of the category. Frequencies of reverse mutations in bacteria were not significantly changed after exposure to the zinc dialkyldithiophosphates. *In vitro* mutation studies in mammalian cells indicate that the zinc dialkyldithiophosphates do not consistently display mutagenic activity in the absence of metabolic activation, however, upon biotransformation, these materials showed mutagenic activity. The findings in bacterial and mammalian cells did not vary in proportion to the alkyl chain length or any other physicochemical parameter.

4.3.2.2.1 Bacterial Gene Mutation Assay

Commercial oil-based samples of four of the twelve substances in this category have been tested in a bacterial reverse mutation test (OECD Guidelines 471 and/or 472). All tested substances were negative for mutagenic activity, with and without metabolic activation.

4.3.2.2.2 Mammalian Gene Mutation Assay in Non-transformed Cells

Commercial oil-based samples of six of the twelve substances in this category were tested in an *in vitro* point mutation assay in mouse embryo cells

(Schechtman and Kouri, 1977[10]). The results of the studies performed in the absence of hepatic microsome activation were inconsistent, but in general indicating that zinc dialkyldithiophosphates have mutagenic potential (3 studies negative, 3 studies positive in the absence of metabolic activation). However, the weight of evidence (2 studies positive, 1 study negative) indicates that metabolic activation of zinc dialkyldithiophosphates by induced hepatic microsomal enzymes results in a significant increase in the mutagenic potential of this class of chemical substances.

4.3.2.2.3 Mammalian Gene Mutation Assay in Transformed Cells

Commercial oil-based samples of five of the twelve substances in this category were tested in an *in vitro* mouse lymphoma cell mutagenicity assay (Guideline 476, *In vitro Mammalian Cell Gene Mutation Test*). The results of these studies indicate that, in the absence of hepatic microsome activation, zinc dialkyldithiophosphates are not mutagenic. However, the weight of evidence indicates that metabolic activation of zinc dialkyldithiophosphates by induced hepatic microsomal enzymes results in a significant increase in the mutagenic potential of this class of chemical substances.

4.3.2.2.4 *In vivo* Chromosomal Aberration Assays

Commercial oil-based samples of four of the twelve substances in this category were tested in an *in vivo* chromosomal aberration assay (OECD Guideline 474, *Mammalian Erythrocyte Micronucleus Test*). All test substances were negative for clastogenicity.

4.3.2.3 Data Assessment and Test Plan for Mutagenicity

Commercial samples for seven of the twelve zinc dialkyldithiophosphates in highly refined lubricating base oil category have been evaluated for genotoxic potential in tests for gene mutations and chromosomal aberrations. The assays included point mutations in bacteria, two types of cultured mammalian cells, and *in vivo* chromosomal aberrations in mice. The findings from the bacterial reverse mutation assay and *in vivo* mouse micronucleus tests were negative for mutagenic potential for all of the tested materials with alkyl side chains that ranged from mixed C3-C6 to C8. The results from the *in vitro* mammalian cell gene mutation test gave inconsistent results for four members of the category with alkyl side chains that ranged from mixed C4-C8. The results from the *in vitro* BALB/3T3 point mutation (ouabain locus) and cell transformation assays indicate that zinc dialkyldithiophosphates may have genotoxic potential. Despite the high cytotoxicity, variability and mixed test results, the overall data indicated that microsome-activated zinc dialkyldithiophosphates were mutagenic. However,

[10] Schechtman LM and Kouri RE. (1977) Control of benzo(a)pyrene-induced mammalian cell cytotoxicity, mutagenicity and transformation by exogenous enzyme fractions. In: Progress in Genetic Toxicology, D. Scott, B.A. Bridges and F.J. Sobels, eds. Elsevier/North-Holland Biomedical Press, New York, pp. 307-316.

zinc ion has been shown to cause cytotoxicity and mutagenicity in similar cultured mammalian cell systems (Amaker et al., 1979[11]). Therefore, as a follow-up to this report, two materials containing zinc (zinc chloride and zinc oleate) were tested in the BALB 3T3 point mutation and cell transformation assays. These two zinc salts were found to be mutagenic in these systems. Further, no mutagenic activity was attributed to a calcium analog of a zinc dialkyldithiophosphate that had previously shown activity in these *in vitro* mammalian cell assays. These findings point to zinc ion as the causative subcomponent in the *in vitro* mammalian cell studies. However, genotoxicity studies conducted in a variety of test systems have failed to provide unequivocal evidence for mutagenicity of zinc (ATSDR, 1992[12]). Furthermore, the US Food and Drug Administration (1982[13]) concluded that zinc ion is not carcinogenic. Dermal carcinogenicity tests conducted in mice revealed that new motor oils containing between 1% and 3% zinc dialkyldithiophosphate were found to be non-carcinogenic (American Petroleum Institute). In summary, the weight of evidence supports the conclusion that zinc dialkyldithiophosphates have a low potential genotoxicity, and that these substances do not present a significant risk for mutagenicity or carcinogenicity in humans.

Bridging will be used to fill the genetic toxicity data gaps for the remaining twelve substances.

Bacterial gene mutation and *in vivo* chromosomal aberration data for commercial samples of the mixed isopropyl and 1,3-dimethylbutyl derivative in highly refined lubricating base oil will be bridged to:

- mixed isobutyl and pentyl derivative.

Bacterial gene mutation and *in vivo* chromosomal aberration data for commercial oil-based samples of the mixed sec-butyl and 1,3-dimethylbutyl derivative and the mixed isobutyl and 2-ethylhexyl derivative will be bridged to:

- mixed sec-butyl and isooctyl derivative
- mixed isobutyl, pentyl, and isooctyl derivative.

Bacterial gene mutation and *in vivo* chromosomal aberration data for a commercial sample of the 2-ethylhexyl derivative in highly refined lubricating base oil will be bridged the remaining members of the category with longest alkyl chain lengths:

- Isooctyl derivative
- Diisodecyl derivative
- Dodecyl phenol derivative

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[11] Amacher et al. Mammalian Cell Mutagenesis: Maturation of Test Systems. Banbury Report 2, 277-293, 1977

[12] Toxicological Profile for Zinc. Prepared by Syracuse Research Corporation and Clement International Corporation for the United States Department of Health and Human Service, Public Health Service, Agency for Toxic Substances and Disease Registry, October 1992

[13] 47 FR 47441 October 1982, corrected 48 FR 3381 January 1983

- Tetrapropenyl phenol derivative

By bridging these data to those substances which lack bacterial gene mutation and *in vivo* chromosomal aberration data, the genetic toxicity of the category has been evaluated with respect to all mutagenic and clastogenic endpoints, and no additional genetic toxicity testing is proposed for the HPV Challenge Program.

4.3.3 Repeated-dose Toxicity of the Zinc Dialkyldithiophosphate Category

4.3.3.1 Repeated-dose Toxicity Test Methodology

Repeated-dose toxicity studies evaluate the systemic effects of repeated exposure to a chemical over a significant period of the life span of an animal (rats, rabbits, or mice). Chronic repeated-dose toxicity studies are concerned with potential adverse effects upon exposure over the greater part of an organism's life span (e.g., one to two years in rodents). Subchronic repeated-dose studies are also concerned with effects caused by exposure for an extended period, but not one that constitutes a significant portion of the expected life span. Subchronic studies are useful in identifying target organ(s), and they can be used in selecting dose levels for longer-term studies. Typically, the exposure regimen in a subchronic study involves daily exposure (at least 5 consecutive days per week) for a period of at least 28 days or up to 90 days (i.e., 4 to 13 weeks). A recovery period of two to four weeks (generally included in most study designs) following completion of the dosing or exposure period provides information on whether or not the effects seen during the exposure period are reversible upon cessation of treatment. The dose levels evaluated in repeated-dose toxicity studies are notably lower than the relatively high limit doses used in acute toxicity studies. The NOAEL (no observed adverse effect level), usually expressed in mg/kg/day, defines the dose of test material that produces no significant toxicological effects. If the test material produces toxicity at the lowest dose tested (i.e., there is no defined NOAEL), the lowest dose that produced an adverse effect is defined as the LOAEL (lowest observed adverse effect level). While these studies are designed to assess systemic toxicity, the study protocol can be modified to incorporate evaluation of potential adverse reproductive and/or developmental effects.

Reproductive and developmental toxicity studies generate information on the effects of a test substance on male and female reproductive performance such as gonadal function, mating behavior, conception, and development of the conceptus, parturition, and post-partum development of the offspring. Various study designs exist, but they all involve exposure to both male and female test animals before mating. The rat is most often selected as the test species. The test substance is administered to males and females continuously at several graduated doses for at least two weeks prior to mating and until the animals are sacrificed. The males are treated for at least two more weeks. Male gonadal histopathology is carefully assessed at the end of the study. The females are treated through parturition and early lactation. The adult females and offspring are typically studied until termination on post-natal day 21, or sometimes earlier. In addition to providing data on fertility and reproduction, this study design provides information on potential developmental toxicity following prenatal and limited post-natal exposure to the test substance. A NOAEL or LOAEL is also used to describe the results of these tests, with the exception that these values are derived from effects specific to reproduction or development.

4.3.3.2 Summary of Repeated-Dose Toxicity Data

A summary of the results from the repeated-dose studies for commercial samples of zinc dialkyldithiophosphates in highly refined lubricating base oil is presented in Table 8. Repeated-dose toxicity tests have been performed on six members of the zinc dialkyldithiophosphate category by two routes of administration and in two species of laboratory animals.

4.3.3.2.1 Systemic Toxicity Tests

Six of the 12 substances in the zinc dialkyldithiophosphate category have been tested for subchronic toxicity.

Commercial oil-based samples of 1,3-dimethyl butyl derivative (CAS # 2215-35-2), mixed sec-butyl and isooctyl derivative (CAS # 113706-15-3), mixed isobutyl and 2-ethylhexyl derivative (CAS # 26566-95-0), mixed isobutyl, pentyl and isooctyl derivative (CAS # 68988-46-5), and 2-ethylhexyl derivative (CAS # 4295-15-8) were each evaluated in separate 21-28 day repeated-dose dermal toxicity studies in rabbits (methodologies consistent with OECD Guideline 410, *Repeated Dose Dermal Toxicity: 21/28 Day*). The concentration of test articles applied to the skin in these studies ranged from 3-100%. Deaths were common at the higher concentrations but the incidence decreased in proportion to a reduction in dose. The clinical signs throughout the treated groups included ano-genital staining, nasal and ocular bloody discharge, lacrimation, diarrhea, lethargy, anorexia, adipsia, loss of body weight, emaciation, and behavioral distress. Moderate-to-severe dermatitis (erythema, edema, atonia, desquamation, fissuring, eschar formation and exfoliation) at the site of topical application was observed in all the treated animals and to a lesser degree in control animals exposed to vehicle. The incidence and severity was proportional to the concentration and duration of exposure to the test material. Significant reductions in hemoglobin, hematocrit and erythrocyte counts were noted in test material treated groups. In addition, several clinical chemistry parameters (alkaline phosphatase, BUN, bilirubin, albumin and cholesterol) were affected by treatment with the test material. Testes and epididymal weights were markedly reduced in the high dose groups and to a lesser degree after lower doses. Adrenal and kidney weights were elevated in some higher dose groups. Microscopic examination of the testes revealed aspermatogenesis, diffuse tubular hypoplasia and reduced mitotic activity. In no dermal study was a NOAEL for systemic toxicity established.

The 2-ethylhexyl derivative (CAS # 4295-15-8) in highly refined lubricating base oil was evaluated in a 28-day repeated dose oral toxicity study in rats (OECD Guideline 407, *Repeated Dose 28-Day Oral Toxicity Study in Rodents*). The test material was administered to rats by oral gavage at 10, 50, 125, 250 and 500 mg/kg/day for 28 consecutive days. Three animals of each sex died at the high dose. One female died at 125 mg/kg/day. Clinically significant findings related to the test material included rales, salivation, and reductions in body weight gain. Necropsy findings included thickened mucosa of the non-glandular mucosa of the stomach in the mid and high dose animals accompanied by microscopic evidence of submucosal edema and suppurative inflammation. Adrenal weights were increased in the high dose animals without evidence of histopathologic abnormalities. The NOAEL was established at 10 mg/kg/day.

4.3.3.2.2 Reproduction and Developmental Toxicity Tests

The 2-ethylhexyl derivative (CAS # 4295-15-8) in highly refined lubricating base oil was tested for reproduction and developmental toxicity (OECD Guideline 421 *Reproduction/ Developmental Screening Test*). The test material was administered to rats by oral gavage at doses of 30, 100 and 200 mg/kg/day. Male and female rats in each dose group received daily treatment for 14 days prior to, and during, the mating period. In addition, the females were treated during gestation and through day 4 of lactation. Control animals received corn oil. Results. Treatment-related deaths (2/12 males, 3/12 females) were recorded in the high dose group. Clinical signs in the decedents included respiratory distress, salivation, hunched posture and mucoid diarrhea. At necropsy, gastric irritation was also observed in the decedents. Mean body weight gain was found to be significantly reduced in the high dose group only. Apart from the gastric observations in the high dose decedents, there were no significant organ weight or microscopic changes because of treatment. There were no significant treatment related effects on reproductive indices or microscopic anatomy of the reproductive organs in the parents of any group. Pup viability in the mid and high dose groups was reduced at parturition and in the post-natal period. The toxicological significant of this finding was questionable. No treatment related effects were observed upon necropsy of the pups found dead or at the scheduled termination. The NOAEL was determined to be 30 mg/kg/day for the parental animals (mortality, clinical signs) and 30 mg/kg/day for the F1 offspring (neonatal mortality).

4.3.3.3 Data Assessment and Test Plan for Repeated-dose Toxicity

Adequate data for repeated-dose toxicity exist for commercial oil-based samples of six of the twelve substances in the zinc dialkyldithiophosphate category. The results of these studies indicate that in repeated dermal exposure to these materials in rabbits can cause moderate-to-severe dermatitis, significant loss of body weight, behavioral distress, reductions in hematology parameters, loss of normal testicular function, and even death at the higher doses. These effects were observed across several members of the category with carbon chain lengths ranging from C4-C8. There was no evidence that the incremental increase in carbon chain length or molecular weight could be correlated with significant changes in toxicity parameters.

Repeated oral administration of commercial samples of zinc dialkyldithiophosphates in highly refined lubricating base oil resulted in evidence of severe gastrointestinal irritation with submucosal edema and suppurative inflammation, mucoid diarrhea, significant body weight loss, distress, and death at the higher doses. However, no significant adverse effects were noted on the testes or male accessory reproductive organs.

The totality of the repeated dose toxicity data indicates that the reproductive organ effects observed in male rabbits are attributed to the stress associated with the severe dermal responses to the test material, rather than direct a systemic response to the test materials. Changes in male reproductive organs in the rabbit have been observed when other irritating substances are applied to the skin at dose levels that cause skin lesions.[14,15] Thus, dermal irritation alone, or in combination with the accompanying weight loss and stress, is thought to play a role in the reproductive organ response to repeated cutaneous application of zinc dialkyldithiophosphates.

4.3.3.4 Repeated-dose toxicity bridging arguments

- Data for the six zinc dialkyldithiophosphates in highly refined lubricating base oil with carbon chain lengths ranging from C4-C8 indicate similar effects, largely attributable to profound dermal irritation at the site of test material application. There was no evidence of increasing toxicity that could be correlated with an increase or decrease in carbon chain length or molecular weight. Consequently, available repeated dose data on the six intermediate carbon chain length zinc dialkyldithiophosphates in highly refined lubricating

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[14]Wong, Z. A., VonBurg, R., Spangler, W. L., and MacGregor, J. A. (1982) Testicular Damage in the Rabbit Resulting from Simple Chemical Cutaneous Irritation. The Toxicologist 2: 41.
[15]McKee, R. H., Kapp, Jr., R. W., and Ward, D. P. (1985) Evaluation of the Systemic Toxicity of Coal Liquefaction-Derived Materials Following Repeated Dermal Exposure in the Rabbit. J. App. Toxicol. 5: 345-351.

base oil will be used as read across to the following category members of similar or higher carbon chain length (molecular weight):

- Mixed isobutyl and pentyl derivative (CAS # 68457-79-4),
- Mixed sec-butyl and 1,3-dimethylbutyl derivative (CAS # 68784-31-6),
- Diisodecyl derivative (CAS # 25103-54-2),
- Dodecylphenol derivative (CAS # 54261-67-5), and
- Tetrapropenylphenol derivative (CAS # 11059-65-7)

4.3.3.5 Repeated-dose reproduction/developmental toxicity bridging arguments

- Reproduction/developmental toxicity data was available for a commercial sample of the 2-ethylhexyl derivative in highly refined lubricating base oil. Although the repeated dose dermal toxicity studies indicated that exposure to zinc dialkyldithiophosphates can have adverse effects on testicular function, it is believed that these effects are secondary to profound dermal irritation, body weight loss and stress. This is evidenced by the lack of reproductive organ effects in the oral reproduction/developmental toxicity assay observed with the 2-ethylhexyl derivative and supportive literature linking dermal irritation and testicular function in experimental animals. Furthermore, epidemiological studies support the lack reproductive findings in experimental analyses. In 1980, National Institute for Occupational Safety and Health investigators conducted a survey to obtain process and toxicology research information, and to conduct employee interviews at a zinc dialkyldithiophosphate production plant[16]. The plant produced zinc dialkyldithiophosphates of various chain lengths ranging from C3-C8. Review of medical histories showed no significant difference between the "exposed" and "controls" zinc dialkyldithiophosphate manufacturing plant workers with regard to birth defects in offspring, or infertility, miscarriages and stillbirths experienced by wives or partners. Physical examination showed no gross abnormalities in secondary sexual characteristics for exposed and controls. Semen analysis showed no azoospermia or oligospermia in the exposed group. Other parameters of the semen analysis showed no significant difference between exposed and controls. The conclusion of the report was that workers exposed to zinc dialkyldithiophosphates in an occupational setting did not exhibit untoward effects on reproductive health when compared to workers not exposed to such compounds.

  Given this information, it is reasonable that the results on the epidemiological study on workers in a zinc dialkyldithiophosphate (C3-C8) manufacturing plant in combination with reproduction/developmental toxicity study results on the 2-ethylhexyl derivative (CAS # 4295-15-8) be used as read-across to the following members of the category:

[16] NIOSH Health Hazard Evaluation Report HETA 80-228-1241, 1980.

- Mixed isopropyl and 1,3-dimethylbutyl derivative (CAS # 84605-29-8)
- Mixed isobutyl and pentyl derivative (CAS # 68457-79-4)
- Mixed sec-butyl and 1,3-dimethylbutyl derivative (CAS # 68784-31-6)
- Mixed sec-butyl and isooctyl derivative (CAS # 113706-15-3)
- Mixed isobutyl and 2-ethylhexyl derivative (CAS # 26566-95-0)
- 1,3-dimethylbutyl derivative (CAS # 2215-35-2)
- Mixed isobutyl, pentyl and isooctyl derivative (CAS # 68988-46-5)
- Isooctyl derivative (CAS # 28629-66-5)
- Diisodecyl derivative (CAS # 25103-54-2),
- Dodecylphenol derivative (CAS # 54261-67-5), and
- Tetrapropenylphenol derivative (CAS # 11059-65-7).

The process of analyzing the existing zinc dialkyldithiophosphate data was performed in a thoughtful and qualitative manner. HERTG concludes that given the relatively minor structural variation between adjacent members of the category, in combination with the totality of human experience and experimental evidence that the reproduction/developmental hazard profile is sufficiently described for the entire category. Therefore, no additional repeated dose toxicity testing is proposed.

The proposal for no additional repeated-dose testing is based on the following considerations.

1) Through the collaborative efforts of the lubricant additive manufacturers, zinc dialkyldithiophosphates are perhaps the most extensively reviewed and tested additive component from a health and safety perspective. The industry has several decades of zinc dialkyldithiophosphate use in automotive crankcases without any evidence of repeated dose or cumulative effects on humans.

2) As discussed above, there is no evidence of direct effects of repeated doses of zinc dialkyldithiophosphates on reproduction systems or indices. Neonatal mortality in rodents following repeated dosing was observed only in the presence of material toxicity, and thus was considered to be of equivocal toxicological significance. Additional animal testing would not significantly contribute to the understanding of the effect of repeated dose exposure to humans, and is unlikely to demonstrate any additional risk to those in the workplace or to the general public.

3) The low potential for repeated dose toxicology in humans is due, in part, to the physical-chemical characteristics of these materials. Zinc dialkyldithiophosphates are high molecular weight components (average > 500 gm/mol), which generally accepted that the molecular weight limit for passive transport across biological membranes. Thus, upon exposure it is unlikely that significant amounts of these components will be absorbed for

systemic distribution. In addition, these materials have a low water solubility that further inhibits absorption and distribution in the mammalian system. A Japanese MITI publication[17] cited a bioaccumulation factor of less than 100 for a C4-C5 ester zinc dithiophosphate indicating a low potential for bioconcentration or cumulative effects. Finally, the negligible vapor pressure and high viscosity at ambient temperature indicates that these materials are unlikely to represent an inhalation exposure under conditions of use.

The exposure profile of zinc dialkyldithiophosphate also demonstrates that there is minimal risk for repeated dose toxicity. Zinc dialkyldithio-phosphates have a singular use in automotive crankcase lubricant additive. Apart from the filling operation, zinc dialkyldithiophosphates are retained in a closed system (i.e., the crankcase), and their use does not result in wide distribution of this component. Furthermore, these materials are designed to undergo thermal decomposition in the crankcase, resulting in the production of a lubricant film on critical engine parts to minimize engine wear and oxidation. Three populations that are most likely to have exposure to zinc dialkyldithiophosphate include manufacture, blending, original equipment manufacturer and downstream automobile service operations. Manufacture of zinc dialkyldithiophosphates is conducted in closed reaction vessels and transfers are performed in closed pipes. The exposure profile to production workers is very low due to process, engineering and personal protection equipment controls. The only practical exposure of concentrated component would be acute and only occur in the rare case of an accidental spill. The dermal route would be the principal means of exposure. Oral or inhalation exposure is expected to be rare.

As mentioned previously, epidemiological studies on workers in zinc dialkyldithiophosphate manufacturing plants did not reveal evidence of cumulative toxicity. Transportation of zinc dialkyldithiophosphates occurs in bulk and transfer to blending operation occurs in closed pipes and vessels. Again, the exposure profile during blending is very low. Zinc dialkyldithiophosphates are then mixed with other lubricant additives, in the presence of high molecular weight, highly refined mineral oil for use as motor oils. The typical concentration of zinc dialkyldithiophosphate in passenger car motor oil is low (e.g., 1-3%). Although OEM factory fill is largely automated and does not result in human contact, dermal exposure to low levels of zinc dialkyldithiophosphate in fresh motor oil is possible for workers in services stations and with do-it-yourself motor oil changers. However, even this small amount of exposure to the general public is falling due to extended drain intervals and the ever increasing fee-for-service lubrication operations staffed by service personnel trained in good occupational hygiene.

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[17] Handbook of Existing and New Chemical Substances. Fifth Edition. Edited by the Chemical Products Safety Division, Basic Industries Bureau, Ministry of International Trade and Industry. Published by Japan Chemical Industry Ecology-Toxicology & Information Center. The Chemical Daily Co. 1992.

4) In addition to the arguments outlined above, HERTG believes that additional testing of zinc dialkyldithiophosphates will cause unnecessary distress to experimental animals.

   Zinc dialkyldithiophosphates are prepared from strong acids that are subsequently neutralized with zinc oxide. Extensive experimental studies demonstrate that zinc dialkyldithiophosphates are skin, eye and mucosal irritants. These hazards are clearly communicated on supplier material safety data sheets (MSDS) and product shipping labels. Animals used in the subchronic dermal toxicity studies were clearly in distress resulting from the severe local skin damage caused by repeated topical administrations of zinc dialkyldithiophosphate. The clinical signs/symptoms along with the supporting gross and microscopic pathology indicate that the experimental animals in the dermal studies experienced distress and suffering. Observations to support this assertion include, but are not limited to, changes in the physical appearance (e.g., blood around eyes and nose as well as ano-genital staining suggestive of a stress- or pain-related condition resulting in secretions not being removed by grooming), and changes in body weight and emaciation (often related changes in food and water consumption due to stress). Furthermore, it is clear that oral administration of zinc dialkyldithiophosphates caused gastrointestinal distress. This conclusion is based on the clinical observations which include salivation and hunched posture following dosing, reductions in body weight, mucoid diarrhea, nasal ocular and ano-genital staining as well as pathological indication of severe gastrointestinal irritation with gastric mucosal edema and suppurative inflammation. It is well accepted that strong mucosal irritants can cause pain, suffering and distress resulting from ulceration and cell death in the stomach lining when administered by the oral route. It is important to remember that the principal hazard of zinc dialkyldithiophosphates is their strong irritant property. HERTG shares EPA's commitment to reduce the number of animals needed for testing and to reduce pain and suffering of test animals to the extent that it is practical and scientifically justifiable. Based on a thoughtful scientific review, HERTG would be unable to conduct additional repeated dose testing of zinc dialkyldithiophosphates without imparting unnecessary and substantial distress and suffering to the experimental animal.

Table 1. Members of the Zinc Dialkyldithiophosphate Category

CAS Number	Chemical Name	Simplified Chemical Name
84605-29-8	Phosphorodithioic acid, mixed O,O-bis(1,3-dimethylbutyl and iso-propyl) esters, zinc salts	Mixed isopropyl and 1,3-dimethylbutyl derivative
68457-79-4	Phosphorodithioic acid, mixed O,O-bis(iso-butyl and pentyl) esters, zinc salts	Mixed isobutyl and pentyl derivative
68784-31-6	Phosphorodithioic acid, mixed O,O-bis(sec-butyl and 1,3-dimethylbutyl) esters, zinc salts	Mixed sec-butyl and 1,3-dimethylbutyl derivative
113706-15-3	Phosphorodithioic acid, mixed O,O-bis(sec-butyl and isooctyl) esters, zinc salts	Mixed sec-butyl and isooctyl derivative
26566-95-0	Phosphorodithioic acid, O-(2-ethylhexyl) O-isobutyl ester, zinc salt	Mixed isobutyl and 2-ethylhexyl derivative
68988-46-5	Phosphorodithioic acid, mixed O,O-bis(iso-butyl and isooctyl and pentyl) esters, zinc salts	Mixed isobutyl, pentyl and isooctyl derivative
2215-35-2	Phosphorodithioic acid, O,O-bis(1,3-dimethylbutyl) ester, zinc salt	1,3-dimethylbutyl derivative
4259-15-8	Phosphorodithioic acid, O,O-bis(2-ethylhexyl) ester, zinc salt	2-ethylhexyl derivative
28629-66-5	Phosphorodithioic acid, O,O-bis(isooctyl) ester, zinc salt	Isooctyl derivative
25103-54-2	Phosphorodithioic acid, O,O-diisodecyl ester, zinc salt	Diisodecyl derivative
54261-67-5	Phenol, dodecyl-, hydrogen phosphorodithioate, zinc salt	Dodecylphenol derivative
11059-65-7	Phenol, tetrapropenyl-, hydrogen phosphorodithioate, zinc salt	Tetrapropenylphenol derivative

Table 2. Chemical Structures of Zinc Dialkyldithiophosphates

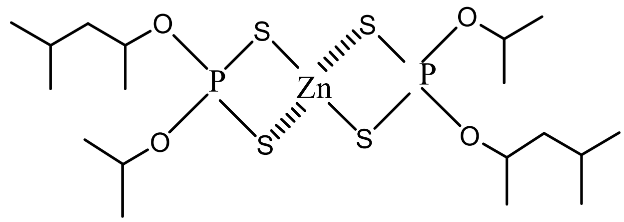
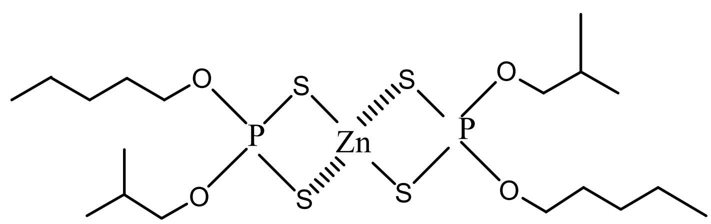
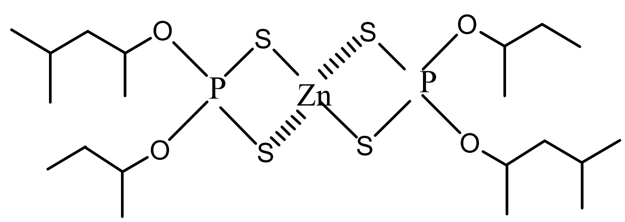
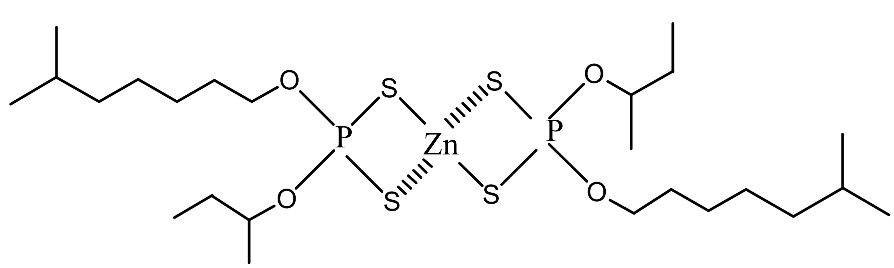
CAS Number	Chemical Structure
84605-29-8	
68457-79-4	
68784-31-6	
113706-15-3	

Table 2. Chemical Structures of Zinc Dialkyldithiophosphates (Cont.)

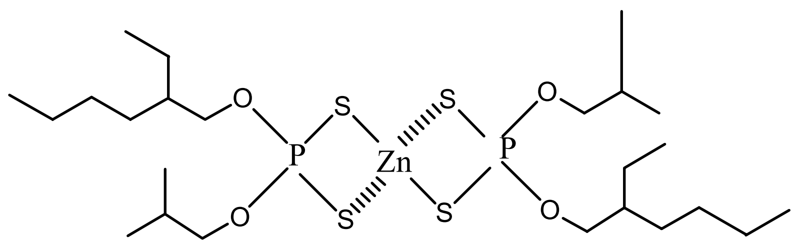
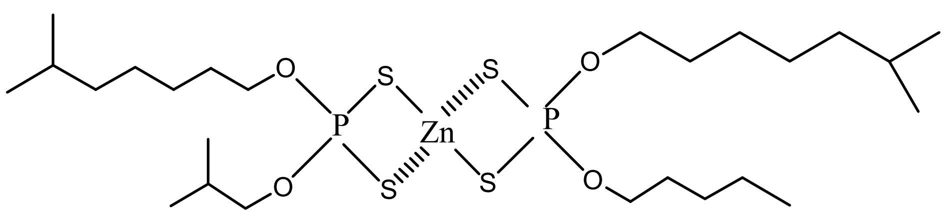
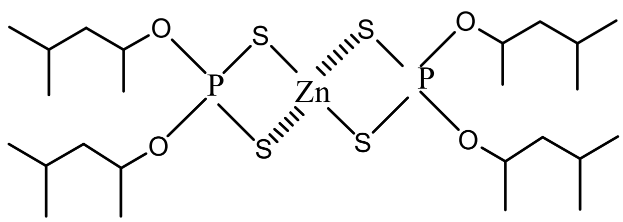
CAS Number	Chemical Structure
26566-95-0	
68988-46-5	
2215-35-2	

Table 2. Chemical Structures of Zinc Dialkyldithiophosphates (Cont.)

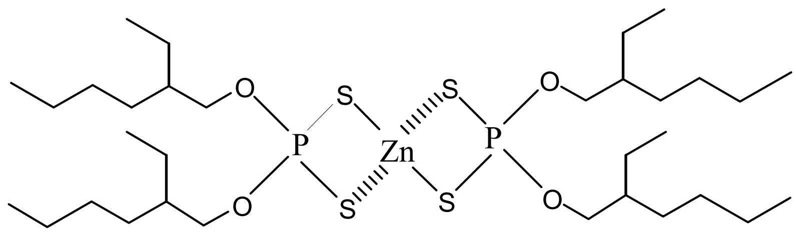
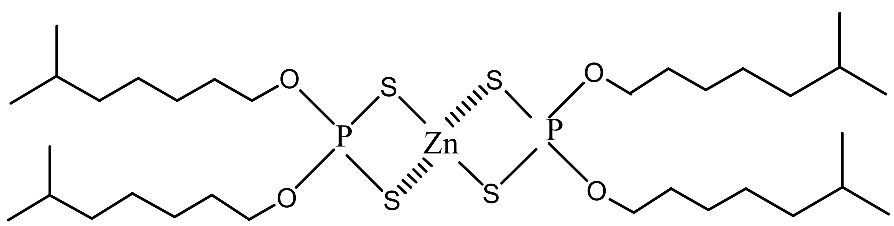
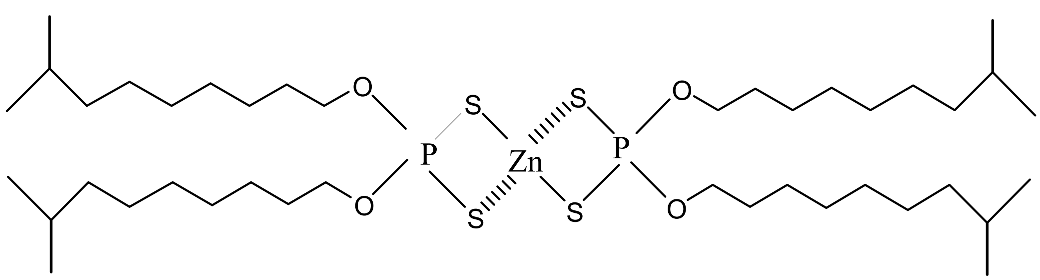
CAS Number	Chemical Structure
4259-15-8	
28629-66-5	
25103-54-2	

Table 2. Chemical Structures of Zinc Dialkyldithiophosphates (Cont.)

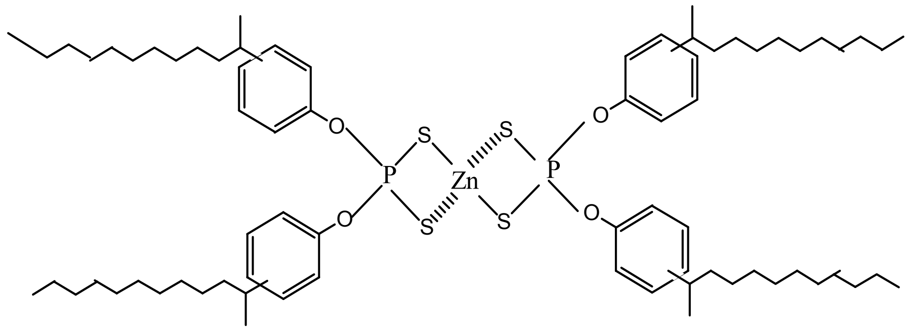
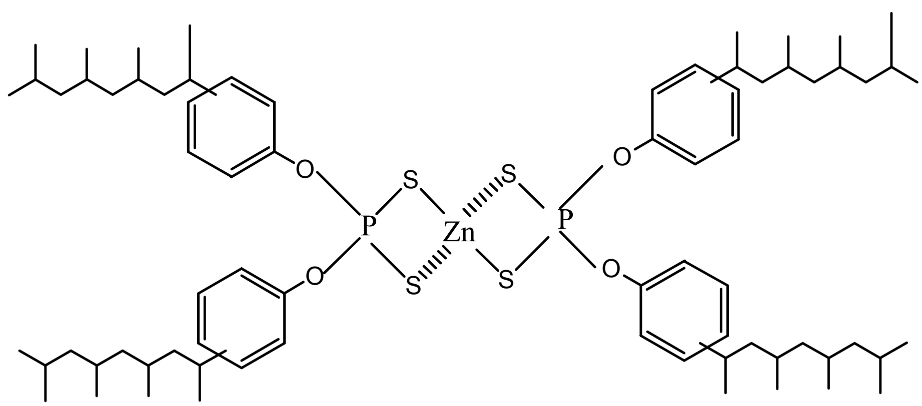
CAS Number	Chemical Structure
54261-67-5	
11059-65-7	

Table 3. Physicochemical Properties of Zinc Dialkyldithiophosphates

CAS Number	Average Molecular Weight (gm/mol)	Alcohol Carbon Number Range	SpecificGravity[2] (gm/ml)	Viscosity[3] (cSt)	Melting Point ^{o}C	Boiling Point ^{o}C	Vapor Pressure[4] (mmHg)	Water Solubility (mg/L)
84605-29-8	578.1	C12-C24	1.145	11.0 @ 100^{o}C	NA	Decomp. @ 120^{o}C	<0.5	Test
68457-79-4	578.1	C16-C20	1.120	115.0 @ 40^{o}C	NA	Decomp. @ 120^{o}C	<0.5	No testing needed Bridging
68784-31-6	606.2	C16-C24	1.080	8.0 @ 100^{o}C	NA	Decomp. @ 120^{o}C	<0.5	No testing needed Bridging
113706-15-3	662.3	C16-C32	No data	No data	NA	Decomp. @ 120^{o}C	<0.5	No testing needed Bridging
26566-95-0	648.3	C16-C32	1.135	12.5 @ 100^{o}C	NA	Decomp. @ 120^{o}C	<0.5	No testing needed Bridging
68988-46-5	634.2	C16-C32	No data	No data	NA	Decomp. @ 120^{o}C	<0.5	No testing needed Bridging
2215-35-2	662.3	C24	No data	No data	NA	Decomp. @ 120^{o}C	<0.5	No testing needed Bridging
4259-15-8	774.5	C32	1.099	15.0 @ 100^{o}C	NA	Decomp. @ 120^{o}C	<0.5	Test
28629-66-5	774.5	C32	No data	No data	NA	Decomp. @ 120^{o}C	<0.5	No testing needed Bridging
25103-54-2	886.7	C40	1.015	18.0 @ 100^{o}C	NA	Decomp. @ 120^{o}C	<0.5	No testing needed Bridging
54261-67-5	1303.3	C72	0.998	30.0 @ 100^{o}C	NA	Decomp. @ 120^{o}C	<0.5	No testing needed Bridging
11059-65-7	1303.3	C72	No data	No data	NA	Decomp. @ 120^{o}C	<0.5	Test

[2]ASTM D1298-99, Standard Test Method for Density, Relative Density (Specific Gravity), or API Gravity of Crude Petroleum and Liquid Petroleum Products by Hydrometer Method
[3]ASTM D 445-97, Standard Test Method for Kinematic Viscosity of Transparent and Opaque Liquids (the Calculation of Dynamic Viscosity)
[4] "De-oiled" zinc dialkyldithiophosphates are solid. Vapor pressure measurements conducted on a C8 ester zinc dialkyldithiophosphate as manufactured (90% in highly refined lubricating base oil), and on a sample of lubricating base oil by itself yielded values of <0.5 mm Hg.
NA – Not applicable for liquids at ambient temperature

Table 4. Evaluation of Environmental Fate Information for Zinc Dialkyldithiophosphates

CAS Number	BIODEGRADABILITY Available Data & Proposed Testing	HYDROLYSIS Available Data & Proposed Testing	PHOTODEGRADATION Available Data & Proposed Testing
84605-29-8	5.9% biodegraded after 28 days	No testing needed	Test (UV Absorption)
68457-79-4	No testing needed Bridging	No testing needed	No testing needed Bridging
68784-31-6	No testing needed Bridging	No testing needed	No testing needed Bridging
113706-15-3	No testing needed Bridging	No testing needed	No testing needed Bridging
26566-95-0	No testing needed Bridging	No testing needed	No testing needed Bridging
68988-46-5	No testing needed Bridging	No testing needed	No testing needed Bridging
2215-35-2	No testing needed Bridging	No testing needed	No testing needed Bridging
4259-15-8	No testing needed Bridging	No testing needed	No testing needed Bridging
28629-66-5	No testing needed Bridging	No testing needed	Test (UV Absorption)
25103-54-2	No testing needed Bridging	No testing needed	No testing needed Bridging
54261-67-5	5.9% biodegraded after 28 days 4.2% biodegraded after 28 days	No testing needed	No testing needed Bridging
11059-65-7	No testing needed Bridging	No testing needed	No testing needed Bridging

Table 5. Evaluation of Aquatic Toxicology of Zinc Dialkyldithiophosphates

CAS Number	ACUTE TOXICITY TO FISH 96-hr LL_{50} (mg/L)[1]	ACUTE TOXICITY TO INVERTEBRATES 48-hr EL_{50} (mg/L)[1]	TOXICITY TO ALGAE 96-hr EL_{50} (mg/L)[1]
	Available Data & Proposed Testing	Available Data & Proposed Testing	Available Data & Proposed Testing
84605-29-8	Test	Test	Test
68457-79-4	No testing needed Bridging	No testing needed Bridging	No testing needed Bridging
68784-31-6	No testing needed Bridging	No testing needed Bridging	No testing needed Bridging
113706-15-3	No testing needed Bridging	No testing needed Bridging	No testing needed Bridging
26566-95-0	No testing needed Bridging	No testing needed Bridging	No testing needed Bridging
68988-46-5	No testing needed Bridging	No testing needed Bridging	No testing needed Bridging
2215-35-2	No testing needed Bridging	No testing needed Bridging	No testing needed Bridging
4259-15-8	Test	Test	Test
28629-66-5	No testing needed Bridging	No testing needed Bridging	No testing needed Bridging
25103-54-2	No testing needed Bridging	No testing needed Bridging	No testing needed Bridging
54261-67-5	No testing needed Bridging	No testing needed Bridging	No testing needed Bridging
11059-65-7	Test	Test	Test

[1]Toxicity endpoints are expressed as median lethal loading rates (LL_{50}) for fish and median effective loading rates (EL_{50}) for *Daphnia* and algae. The EL/LL_{50} is defined as the loading rate that adversely effects 50% of the test organisms exposed to it during a specific time. The greater the EL/LL_{50} the lower the toxicity.
F = fathead minnow, *Pimephales promelas*.
D = freshwater cladoceran, *Daphnia magna*.
P = freshwater algae *Pseudokirchneriella subcapitata* formerly called *Selenastrum capricornutum*.
T = rainbow trout, *Oncorhynchus mykiss* formerly called *Salmo gairdneri.*
S = sheepshead minnow, *Cyprinodon variegatus*.
R = algae growth rate.
B = algae biomass.

Table 6. Evaluation of Acute Mammalian Toxicology of Zinc Dialkyldithiophosphates

CAS Number	ACUTE ORAL TOXICITY[1]	ACUTE DERMAL TOXICITY[1]
	Available Data & Proposed Testing	Available Data & Proposed Testing
84605-29-8	LD_{50} > 2.0 g/kg (rat)	LD_{50} > 2.0 g/kg (rabbit)
68457-79-4	LD_{50} > 2.0 g/kg (rat)	LD_{50} > 5.0 g/kg (rabbit)
68784-31-6	LD_{50} > 2.0 g/kg (rat)	LD_{50} > 5.0 g/kg (rabbit)
113706-15-3	No testing needed Bridging	No testing needed Acute toxicity end point satisfied by acute oral toxicity results
26566-95-0	No testing needed Bridging	No testing needed Acute toxicity end point satisfied by acute oral toxicity results
68988-46-5	No testing needed Bridging	LD_{50} > 2.0 g/kg (rabbit)
2215-35-2	LD_{50} > 2.0 g/kg (rat)	No testing needed Acute toxicity end point satisfied by acute oral toxicity results
4259-15-8	LD_{50} > 2.0 g/kg (rat)	LD_{50} > 5.0 g/kg (rabbit)
28629-66-5	No testing needed Bridging	No testing needed Acute toxicity end point satisfied by acute oral toxicity results
25103-54-2	No testing needed Bridging	LD_{50} > 8.0 g/kg (rabbit)
54261-67-5	No testing needed Bridging	No testing needed Acute toxicity end point satisfied by acute oral toxicity results
11059-65-7	No testing needed Bridging	No testing needed Acute toxicity end point satisfied by acute oral toxicity results

[11]Toxicity endpoints are expressed as median lethal dose (LD_{50}) for acute oral and dermal toxicity.

Table 7. Evaluation of Mutagenicity of Zinc Dialkyldithiophosphates

CAS Number	GENE MUTATION ASSAY Available Data & Proposed Testing	CHROMOSOMAL ABERRATION ASSAY Available Data & Proposed Testing
84605-29-8	• Bacterial Reverse Mutation Assay – Not mutagenic • *In vitro* Point Mutation Assay in Mouse Embryo Cells- Not mutagenic in the absence of metabolic activation • *In vitro* Point Mutation Assay in Mouse Embryo Cells- Mutagenic in the presence of metabolic activation	Mouse Micronucleus Assay – Not clastogenic
68457-79-4	• *In vitro* Point Mutation Assay in Mouse Embryo Cells- Mutagenic in the absence of metabolic activation • *In vitro* Mouse Lymphoma Mutagenicity Assay – Not mutagenic in the absence of metabolic activation	No testing needed Bridging
68784-31-6	Bacterial Reverse Mutation Assay – Not mutagenic	Mouse Micronucleus Assay – Not clastogenic
113706-15-3	• *In vitro* Point Mutation Assay in Mouse Embryo Cells- Mutagenic in the absence of metabolic activation (only at extremely high toxic doses) • *In vitro* Mouse Lymphoma Mutagenicity Assay – Not mutagenic in the absence of metabolic activation • *In vitro* Mouse Lymphoma Mutagenicity Assay – Mutagenic in the presence of metabolic activation	No testing needed Bridging

CAS Number	GENE MUTATION ASSAY	CHROMOSOMAL ABERRATION ASSAY
	Available Data & Proposed Testing	Available Data & Proposed Testing
26566-95-0	• Bacterial Reverse Mutation Assay – Not mutagenic • *In vitro* Point Mutation Assay in Mouse Embryo Cells- Mutagenic in the absence of metabolic activation • *In vitro* Point Mutation Assay in Mouse Embryo Cells- Mutagenic in the presence of metabolic activation • *In vitro* Mouse Lymphoma Mutagenicity Assay – Not mutagenic in the absence of metabolic activation • *In vitro* Mouse Lymphoma Mutagenicity Assay – Equivocal mutagenic response in the presence of metabolic activation	Mouse Micronucleus Assay – Not clastogenic
68988-46-5	• *In vitro* Point Mutation Assay in Mouse Embryo Cells- Not mutagenic in the absence of metabolic activation • *In vitro* Mouse Lymphoma Mutagenicity Assay – Not mutagenic in the absence of metabolic activation • *In vitro* Mouse Lymphoma Mutagenicity Assay – Mutagenic in the presence of metabolic activation	No testing needed Bridging
2215-35-2	No testing needed Bridging	No testing needed Bridging

CAS Number	GENE MUTATION ASSAY	CHROMOSOMAL ABERRATION ASSAY
	Available Data & Proposed Testing	Available Data & Proposed Testing
4259-15-8	• Bacterial Reverse Mutation Assay – Not mutagenic • *In vitro* Point Mutation Assay in Mouse Embryo Cells- Not mutagenic in the absence of metabolic activation • *In vitro* Point Mutation Assay in Mouse Embryo Cells- Mutagenic in the presence of metabolic activation • *In vitro* Mouse Lymphoma Mutagenicity Assay – Not mutagenic in the absence of metabolic activation • *In vitro* Mouse Lymphoma Mutagenicity Assay – Equivocal mutagenic response in the presence of metabolic activation	Mouse Micronucleus Assay – Not clastogenic
28629-66-5	No testing needed Bridging	No testing needed Bridging
25103-54-2	No testing needed Bridging	No testing needed Bridging
54261-67-5	No testing needed Bridging	No testing needed Bridging
11059-65-7	No testing needed Bridging	No testing needed Bridging

Table 8. Evaluation of Repeated-dose Mammalian Toxicology of Zinc Dialkyldithiophosphates

CAS Number	REPEATED-DOSE TOXICITY Available Data & Proposed Testing	REPRODUCTIVE/DEVELOPMENTAL TOXICITY Available Data & Proposed Testing
84605-29-8	No testing needed Bridging	No testing needed Bridging
68457-79-4	No testing needed Bridging	No testing needed Bridging
68784-31-6	No testing needed Bridging	No testing needed Bridging
113706-15-3	28-day dermal toxicity study in rabbits NOAEL not established (adverse effects at all doses) <u>25%</u> • Four deaths or moribund sacrifices • Body weight loss • Erythema, edema, atonia, desquamation, eschar formation and exfoliation • Reduction in hemoglobin, hematocrit and erythrocyte counts • Platelet count elevation • Increased serum cholesterol • Decreased serum albumin • Reduction in plasma, erythrocyte and brain cholinesterase levels • Testes and epididymal weight reduction • Adrenal and kidney weight elevation • Morphological abnormalities in the seminiferous tubules characterized by aspermatogenesis, diffuse tubular hypoplasia and reduced mitotic activity <u>5%</u> • One death • Body weight loss • Erythema, edema, atonia, desquamation, fissuring, eschar formation and exfoliation	No testing needed Bridging

CAS Number	REPEATED-DOSE TOXICITY Available Data & Proposed Testing	REPRODUCTIVE/DEVELOPMENTAL TOXICITY Available Data & Proposed Testing
	• Increased serum cholesterol • Reduction in plasma, erythrocyte and brain cholinesterase levels • Kidney weight elevation Vehicle control • Dermal irritation (lesser degree than in treatment groups)	
26566-95-0	21-day repeated-dose dermal study in rabbits NOAEL not established (adverse effects at all doses) 860 mg/kg/day • One death (male) • Decedent clinical signs included severe dermal reactions, loss of body weight, anorexia, adipsia, diarrhea, lethargy • Survivor clinical signs included moderate-severe dermal reactions, weight loss, nasal and ocular discharge, gastrointestinal distress, occasional lethargy and ptosis, suppression of sperm formation (aspermia) 430 mg/kg/day • Two deaths (male and female) • Decedent clinical signs included severe dermal reactions, loss of body weight, anorexia, adipsia, diarrhea, lethargy • Survivor clinical signs included moderate-severe dermal reactions, weight loss, nasal and ocular discharge, gastrointestinal distress, occasional lethargy and ptosis, suppression of sperm formation 210 mg/kg/day • No deaths • Survivor clinical signs included moderate-severe dermal reactions, weight loss, nasal and ocular discharge,	No testing needed Bridging

CAS Number	REPEATED-DOSE TOXICITY	REPRODUCTIVE/DEVELOPMENTAL TOXICITY
	Available Data & Proposed Testing	Available Data & Proposed Testing
	gastrointestinal distress, occasional lethargy and ptosis Control • Various signs of distress including nasal and ocular discharge, gastrointestinal distress, occasional lethargy and ptosis, • One animal with severely reduced spermatogenesis	

<table>
<tr><th rowspan="2">CAS Number</th><th>REPEATED-DOSE TOXICITY</th><th>REPRODUCTIVE/DEVELOPMENTAL TOXICITY</th></tr>
<tr><th>Available Data & Proposed Testing</th><th>Available Data & Proposed Testing</th></tr>
<tr><td>68988-46-5</td><td>21-day repeated-dose dermal study in rabbits
NOAEL not established (adverse effects at all doses)

<u>100%</u>
• All animals in group died (18)
• Moderate-severe dermal reactions in proportion to dose
• Hyperirritability, diarrhea, decrease motor activity, ataxia, loss of righting reflex, ocular discharge
• Severe body weight losses
• Reductions in hematology parameters
<u>25%</u>
• 15/18 animals died
• Moderate-severe dermal reactions in proportion to dose
• Hyperirritability, diarrhea, decrease motor activity, ataxia, loss of righting reflex, ocular discharge
• Severe body weight losses
• Reductions in hematology parameters
<u>5%</u>
• One death
• Moderate-severe dermal reactions in proportion to dose
• Hyperirritability, diarrhea, decrease motor activity, ataxia, loss of righting reflex, ocular discharge
• Body weight losses
• Reductions in hematology parameters
<u>3%</u>
• No deaths
• Moderate-severe dermal reactions in proportion to dose
• Hyperirritability, diarrhea, decrease motor activity, ataxia, loss of righting reflex, ocular discharge
• Body weight losses</td><td>No testing needed
Bridging</td></tr>
</table>

CAS Number	REPEATED-DOSE TOXICITY Available Data & Proposed Testing	REPRODUCTIVE/DEVELOPMENTAL TOXICITY Available Data & Proposed Testing
	• Reductions in hematology parameters Vehicle control • One death • Moderate-severe dermal reactions in proportion to dose • Body weight losses Sham control • No deaths	
2215-35-2	21-day repeated-dose dermal study in rabbits NOAEL not established (adverse effects at all doses) 1.6 ml/kg/day • Three animals sacrificed moribund • Severe erythema and edema at the site of application • Body weight loss • White blood cell count reductions • Increased serum triglyceride, uric acid, SGOT, LDH and GGT • Reductions in testes, liver, heart and ovary weights • Decreased spermatogenesis 0.8 ml/kg/day • One death • Severe erythema and edema at the site of application • Progressive weight loss over the course of study • White blood cell count reductions Naïve control • Two deaths	No testing needed Bridging
4259-15-8	28-day repeated-dose oral study in rats (OECD 407) NOAEL = 10 mg/kg/day	Reproduction/developmental oral toxicity screening test in rats (OECD 421) NOAEL P0 = 30 mg/kg/day

CAS Number	REPEATED-DOSE TOXICITY	REPRODUCTIVE/DEVELOPMENTAL TOXICITY
	Available Data & Proposed Testing	Available Data & Proposed Testing
	<u>500 mg/kg/day</u> • Four deaths (3 male and 1 female) • Clinical signs included changes in fecal consistency and coloration, staining of body surfaces, rales, salivation, aggressive behavior, reduced food consumption, reduced body weight gain • Necropsy findings included edema, suppurative inflammation and thickening of the mucosa of non-glandular stomach; increase in adrenal weights <u>250 mg/kg/day</u> • No deaths • Clinical signs included changes in fecal consistency and coloration, staining of body surfaces, rales, salivation, aggressive behavior, reduced body weight gain • Necropsy findings included thickened mucosa of non-glandular stomach; increase in adrenal weights <u>125 mg/kg/day</u> • One death (female; not treatment related) • Clinical signs included changes in fecal consistency and coloration, staining of body surfaces, rales, salivation and aggressive behavior <u>50 mg/kg/day</u> • No deaths • Rales and salivation <u>10 mg/kg/day</u> • No significant adverse effects	NOAEL F1 = 30 mg/kg/day Parental animals <u>200 mg/kg/day</u> • Five deaths (two males, three females) • Clinical signs included respiratory distress, hunched appearance and mucoid diarrhea • Reduced pre-mating body weight gain (males) • Necropsy revealed evidence of gastric irritation • No significant effects on reproductive parameters • No microscopic reproductive organ effects <u>100 mg/kg/day</u> • Deaths • Clinical signs included respiratory distress, hunched appearance and mucoid diarrhea • Necropsy revealed evidence of gastric irritation • No significant effects on reproductive parameters • No microscopic reproductive organ effects <u>30 mg/kg/day</u> • No deaths • No significant treatment-related findings • No significant effects on reproductive parameters • No microscopic reproductive organ effects Offspring effects <u>200 mg/kg/day</u> • One incident of total litter loss • Increased pup mortality during post-natal period <u>100 mg/kg/day</u> • Two incidents of total litter loss

CAS Number	REPEATED-DOSE TOXICITY Available Data & Proposed Testing	REPRODUCTIVE/DEVELOPMENTAL TOXICITY Available Data & Proposed Testing
		• Increased pup mortality during post-natal period <u>30 mg/kg/day</u> • No significant adverse effects
28629-66-5	28-day repeated-dose dermal study in rabbits NOEAL not established (adverse effects at all doses) <u>25%</u> • 4/20 animals died • Moderate-to-severe dermal reactions (proportional to dose) • Body weight losses • • Alterations in hematology and clinical chemistry parameters • Testicular hypotrophy and aspermatogenesis <u>5%</u> • No deaths • Moderate-to-severe dermal reactions (proportional to dose) • Body weight losses • • Alterations in hematology and clinical chemistry parameters • Testicular hypotrophy and aspermatogenesis <u>Vehicle control</u> • No deaths •	No testing needed Bridging
25103-54-2	No testing needed Bridging	No testing needed Bridging

CAS Number	REPEATED-DOSE TOXICITY	REPRODUCTIVE/DEVELOPMENTAL TOXICITY
	Available Data & Proposed Testing	Available Data & Proposed Testing
54261-67-5	No testing needed Bridging	No testing needed Bridging
11059-65-7	No testing needed Bridging	No testing needed Bridging

Table 9. Summary Table

CAS Number	Environmental Fate					Ecotoxicity			Human Health Effects				
	Physical Chem	Photodeg	Hydrolysis	Fugacity	Biodeg	Acute Fish Toxicity	Acute Invert Toxicity	Algal Toxicity	Acute Toxicity	Point Mutations	Chrom Effects	Sub-chronic	Repro/ Develop
84605-29-8	T^1	T^2	D	C	A	T	T	T	A	A	A	B	B
68457-79-4	D	B	D	C	B	B	B	B	A	A	B	B	B
68784-31-6	D	B	D	C	B	B	B	B	A	A	A	B	B
113706-15-3	D	B	D	C	B	B	B	B	B	A	B	A	B
26566-95-0	D	B	D	C	B	B	B	B	B	A	A	A	B
68988-46-5	D	B	D	C	B	B	B	B	A	A	B	A	B
2215-35-2	D	B	D	C	B	B	B	B	A	B	B	A	B
4259-15-8	T^1	B	D	C	B	T	T	T	A	A	A	A	A
28629-66-5	D	T^2	D	C	B	B	B	B	B	B	B	A	B
25103-54-2	D	B	D	C	B	B	B	B	A	B	B	B	B
54261-67-5	D	B	D	C	A	B	B	B	B	B	B	B	B
11059-65-7	T^1	B	D	C	B	T	T	T	B	B	B	B	B

A Adequate data available
C Computer modeling proposed
B Bridging
D Technical discussion proposed
T Test
T^1 Solubility Testing
T^2 UV absorption